```
     (FILE 'HOME' ENTERED AT 17:38:04 ON 15 SEP 1999)

     FILE 'CA, BIOSIS, MEDLINE, DRUGU, EMBASE' ENTERED AT 17:38:47 ON 15 SEP
     1999
L1          864 S (TNF OR TUMOR NECROSIS FACTOR?)(2A)ANTAGONIST?
L2       551832 S (NEURO? OR NERV? OR SPINAL CORD OR BRAIN)(2A)(DISEAS? OR
DISO
L3      2764507 S HERNIATED DISC? OR CARPAL TUNNEL SYNDROME OR PITUITARY
ADENOM
L4          829 S L1 AND (L2 OR L3)
L5          381 S L1(10A)(L2 OR L3)
L6          224 DUP REM L5 (157 DUPLICATES REMOVED)
L7          190 S L6 NOT PY>1998
L8       213100 S HIS


=> s herniated disc? or carpal tunnel syndrome or pituitary adenoma? or
intracran? pressur? or lesion? or ms or autoimmun?(2a)(neuro? or nerv?) or
multiple sclero? or panencephalit?

   2 FILES SEARCHED...
L9     1188640 HERNIATED DISC? OR CARPAL TUNNEL SYNDROME OR PITUITARY
ADENOMA?
               OR INTRACRAN? PRESSUR? OR LESION? OR MS OR
AUTOIMMUN?(2A)(NEURO?
                OR NERV?) OR MULTIPLE SCLERO? OR PANENCEPHALIT?

=> s l1(10a)(l2 or l9)

L10          3 L1(10A)(L2 OR L9)

=> s l1 and (l2 or l9)

L11         64 L1 AND (L2 OR L9)

=> dup rem l11

PROCESSING COMPLETED FOR L11
L12          38 DUP REM L11 (26 DUPLICATES REMOVED)

=> d 1-38 bib,ab


L12   ANSWER 1 OF 38  DRUGU  COPYRIGHT 1999 DERWENT INFORMATION LTD
AN    1999-29912  DRUGU   P
TI    Therapeutic potential and strategies for inhibiting tumor necrosis
      factor-alpha.
AU    Newton R C; Decicco C P
CS    Du-Pont
LO    Wilmington, Del., USA
SO    J.Med.Chem. (42, No. 13, 2295-314, 1999) 4 Fig. 249 Ref.
      CODEN: JMCMAR       ISSN:  0022-2623
AV    Department of Inflammatory Diseases Research, The DuPont Pharmaceuticals
      Company,  Experimental Station, P.O. Box 80500, Wilmington, Delaware
      19880-0500, U.S.A.
```

LA English
DT Journal
FA AB; LA; CT
FS Literature
AB Therapeutic potential and strategies for inhibiting TNF-alpha (TNF) are reviewed. Topics covered include: (i) signal transduction and the role of transcription factor NF-kappa-B in TNF expression; (ii) cAMP modulation; in general, agents that increase cAMP inhibit TNF production; (iii) cAMP phosphodiesterase (PDE) inhibition; selective PDE4 inhibitors show antiinflammatory activity in conditions associated with elevated TNF, such as asthma and rheumatoid arthritis; (iv) mitogen-activated protein (MAP) kinase inhibition and regulation of TNF expression; (v) p38 inhibitors and translational regulation of TNF expression; (vi) gene regulation; and (vii) interference with any aspect of TNF-receptor-signaling complex.

L12 ANSWER 2 OF 38 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1999:336297 BIOSIS
DN PREV199900336297
TI Efficacy of Thalidomide in Crohn's disease.
AU Wedel, Susanne (1); Bauditz, J. (1); Suk, A. (1); Lochs, H. (1)
CS (1) Univsitats Klinikum Charite, Berlin Germany
SO Gastroenterology, (April, 1999) Vol. 116, No. 4 PART 2, pp. A843.
Meeting Info.: Digestive Disease Week and the 100th Annual Meeting of the American Gastroenterological Association Orlando, Florida, USA May 16-19, 1999 American Gastroenterological Association
. ISSN: 0016-5085.
DT Conference
LA English

L12 ANSWER 3 OF 38 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1999:92459 BIOSIS
DN PREV199900092459
TI Normalization of creatine kinase level during arthritis in a patient with Becker muscular dystrophy.
AU Maegaki, Yoshihiro (1); Ogura, Kaeko; Maeoka, Yukinori; Takeshita, Kenzo
CS (1) Div. Child Neurol., Inst. Neurol. Sci., Fac. Med., Tottori Univ., 36-1 Nishi-Machi, Yonago 683-8504 Japan
SO Neurology, (Jan. 1, 1999) Vol. 52, No. 1, pp. 172-174.
ISSN: 0028-3878.
DT Article
LA English
AB A patient with Becker muscular dystrophy had transient arthritis. During the active illness his serum creatine kinase (CK) level normalized and serum levels of soluble interleukin 2 (IL-2) receptor, IL-6, IL-1 receptor **antagonist**, and soluble **tumor necrosis factor** receptor 2 were elevated. CK increased to his usual levels after arthritis remission whereas the levels of inflammatory cytokines and their inhibitors decreased.

L12 ANSWER 4 OF 38 CA COPYRIGHT 1999 ACS DUPLICATE 1
AN 131:43372 CA
TI Early neuronal expression of tumor necrosis factor-.alpha. after experimental **brain injury** contributes to **neurological** impairment
AU Knoblach, Susan M.; Fan, Lei; Faden, Alan I.
CS Georgetown Institute for Cognitive and Computational Sciences, Georgetown University Medical Center, Washington, DC, 20007-2197, USA
SO J. Neuroimmunol. (1999), 95(1,2), 115-125
CODEN: JNRIDW; ISSN: 0165-5728

PB Elsevier Science B.V.
DT Journal
LA English
AB Tumor necrosis factor-.alpha. (TNF.alpha.) is a pleiotropic cytokine involved in inflammatory cascades assocd. with CNS injury. To examine the role of TNF.alpha. in the acute pathophysiol. of **traumatic brain injury** (TBI), the authors studied its expression, localization, and modulation in a clin. relevant rat model of non-penetrating head trauma. TNF.alpha. levels increased in the injured cortex at 1 and 4, but not at 12, 24, or 72 h after severe lateral fluid-percussion trauma. TNF.alpha. was not elevated after mild injury. At 1 and 4 h after severe TBI, marked increases of TNF.alpha. were localized immunocytochem. to **neurons** of the **injured** cerebral cortex. A small population of astrocytes, ventricular cells, and microvessels, also showed pos. TNF.alpha. staining, but this expression was not injury-dependent. Macrophages that were present in a hemorrhagic zone along the external capsule, corpus callosum, and alveus hippocampus at 4 h after TBI did not express TNF.alpha.. Intracerebroventricular administration of a selective **TNF**.alpha. **antagonist**, sol. **TNF**.alpha. receptor fusion protein (sTNFR:Fc) (37.5 .mu.g), at 15 min before and 1 h after TBI, improved performance in a series of standardized motor tasks after injury. In contrast, i.v. administration of sTNFR:Fc (0.2, 1, or 5 mg/kg) at 15 min after trauma did not improve motor outcome. Collectively, this evidence suggests that enhanced early neuronal expression of TNF.alpha. after TBI contributes to subsequent neurol. dysfunction.

L12 ANSWER 5 OF 38 CA COPYRIGHT 1999 ACS DUPLICATE 2
AN 130:294617 CA
TI Pathophysiological role of the cytokine network in the anterior pituitary gland
AU Arzt, Eduardo; Pereda, Marcelo Paez; Castro, Carolina Perez; Pagotto, Uberto; Renner, Ulrich; Stalla, Gunter K.
CS Laboratorio de Fisiologia y Biologia Molecular. Dept. de Biologia, Universidad de Buenos Aires, Buenos Aires, Argent.
SO Front. Neuroendocrinol. (1999), 20(1), 71-95
CODEN: FNEDA7; ISSN: 0091-3022
PB Academic Press
DT Journal; General Review
LA English
AB A review, with 178 refs. Recent evidence has demonstrated that cytokines and other growth factors act in the anterior pituitary gland. Using the traditional criteria employed to det. autocrine or paracrine functions our review shows that, in addn. to their role as lymphocyte messengers, certain cytokines are autocrine or paracrine regulators of anterior pituitary function and growth. The cytokines known to regulate and/or be expressed in the anterior pituitary include the inflammatory cytokine family (IL-1 and its endogenous **antagonist**, IL-1ra; **TNF** -.alpha., and IL-6), the Th1-cytokines (IL-2 and IFN-.gamma.), and other cytokines such as LIF, MIF, and TGF-.beta.. This review examines at the cellular, mol., and physiol. levels whether: (1) each cytokine alters some aspect of pituitary physiol.; (2) receptors for the cytokine are expressed in the gland; and (3) the cytokine is produced in the anterior pituitary. Should physiol. stimuli regulate pituitary cytokine prodn., this would constitute addnl. proof of their autocrine/paracrine role. In this context, we analyze in this review the current literature on the actions of cytokines known to regulate anterior pituitary hormone secretion, selecting the in vivo studies that support the direct action of the cytokine in the anterior pituitary. Further support for direct regulatory

action is provided by in vitro studies, in explant cultures or pituitary cell lines. The cytokine receptors that have been demonstrated in the pituitary of several species are also discussed. The endogenous prodn. of the homologous cytokines and the regulation of this expression are analyzed. The evidence indicating that cytokines also regulate the growth and proliferation of pituitary cells is reviewed. This action is particularly important since it suggests that intrinsically produced cytokines may play a role in the pathogenesis of **pituitary adenomas**. The complex cell to cell communication involved in the action of these factors is discussed. (c) 1999 Academic Press.

L12 ANSWER 6 OF 38 CA COPYRIGHT 1999 ACS
AN 130:29195 CA
TI Therapeutic suppression of tumor necrosis factor-.alpha. and vascular endothelial growth factor
IN Feldmann, Marc; Maini, Ravinder Nath; Paleolog, Ewa Maria
PA The Kennedy Institute of Rheumatology, UK
SO PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9851344	A1	19981119	WO 1998-GB1343	19980512
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9873457	A1	19981208	AU 1998-73457	19980512

PRAI US 1997-854881 19970512
WO 1998-GB1343 19980512
AB Methods for treating and/or preventing a TNF-mediated disease in an individual are disclosed. Also disclosed are compns. comprising a **TNF**.alpha. **antagonist** and a VEGF antagonist. TNF-mediated diseases include rheumatoid arthritis, Crohn's disease, and acute and chronic immune diseases assocd. with transplantation.

L12 ANSWER 7 OF 38 CA COPYRIGHT 1999 ACS
AN 129:40158 CA
TI Suppression of TNF.alpha. and IL-12 in therapy
IN Feldmann, Marc; Malfait, Anne-Marie Aline Michel; Butler, Debra Maree; Brennan, Fionula Mary; Maini, Ravinder Nath
PA Kennedy Institute of Rheumatology, UK; Feldmann, Marc; Malfait, Anne-Marie Aline Michel; Butler, Debra Maree; Brennan, Fionula Mary; Maini, Ravinder Nath
SO PCT Int. Appl., 66 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9822137	A1	19980528	WO 1997-GB3151	19971117
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG,				

US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9749599 A1 19980610 AU 1997-49599 19971117
EP 936923 A1 19990825 EP 1997-912367 19971117
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
PRAI US 1996-749979 19961115
WO 1997-GB3151 19971117
AB Methods for treating and/or preventing a TNF.alpha.-mediated disease in an individual are disclosed. Also disclosed are compns. comprising a **TNF antagonist** and an IL-12 antagonist. The **TNF** .alpha. **antagonist** is an antibody or a TNF receptor/IgG fusion protein or thalidomide, and the IL-12 antagonist is an antibody or phosphodiesterase inhibitor, e.g. pentoxifylline or rolipram. TNF.alpha.-mediated diseases include rheumatoid arthritis, Crohn's disease, and acute and chronic immune diseases assocd. with transplantation.

L12 ANSWER 8 OF 38 DRUGU COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1998-42380 DRUGU C P B S
TI Novel cyclic compounds as potent phosphodiesterase 4 inhibitors.
AU He W; Huang F C; Hanney B; Souness J; Miller B; Liang G; Mason J; Djuric S
CS Rhone-Poulenc-Rorer
LO Collegeville, Pa., USA
SO J.Med.Chem. (41, No. 22, 4216-23, 1998) 5 Fig. 5 Tab. 18 Ref.
CODEN: JMCMAR ISSN: 0022-2623
AV Department of Medicinal Chemistry, SW 8 Rhone-Poulenc Rorer Centra Research, 500 Arcola Road, Collegeville, PA, U.S.A.
LA English
DT Journal
FA AB; LA; CT
FS Literature
AB A series of 2,2-disubstituted indan-1,3-diones was prepared, designed as phosphodiesterase 4 (PDE4) inhibitors, using RP-73401 (2) as lead compound. Structure-activity relationships were evaluated. Compounds were tested for pig macrophage PDE4 inhibition, binding at 3H-rolipram sites in guinea pig brain, inhibition of TNF-alpha release from human monocytes, and p.o. inhibition of TNF-alpha release in mice with endotoxemia, using (2) as standard. Unlike classical PDE4 inhibitors, i.v. analogs were nonemetic in dogs. The wide range of catalytic/rolipram affinity ratios challenged the hypothesis regarding the role of selectivity and the catalytic/rolipram binding sites in their contribution to toxicity.

L12 ANSWER 9 OF 38 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1999:149448 BIOSIS
DN PREV199900149448
TI In vitro inhibition of binding of tumor necrosis factor (TNF)-alpha by monoclonal antibody to TNF receptor on glioma cell and monocyte.
AU Tanaka, Satoshi (1); Nagashima, Tadashi; Hori, Tomokatsu
CS (1) Div. Neurosurgery, Inst. Neurological Sciences, Faculty Med., Tottori Univ. Sch. Med., 36-1 Nishi-cho, Yonago, Tottori 683-8504 Japan
SO Neurologia Medico-Chirurgica, (Dec., 1998) Vol. 38, No. 12, pp. 812-818. ISSN: 0387-2572.
DT Article
LA English
AB The use of monoclonal antibodies to the tumor necrosis factor (TNF) receptors, the TNF-p55 receptor (TNF-p55R) and the TNF-p75 receptor (TNF-p75R), was evaluated to reduce the effects of TNF caused by binding to TNF-p75R. Competitive binding of anti-TNF-p55R (mAbp55R) and

anti-TNF-p75R monoclonal antibodies (mAbp75R) with iodine-125-labeled TNF-alpha to GL-9 glioma cells and U937 histiocytic lymphoma cells was evaluated. The effects of mAbp55R and mAbp75R on the growth suppression by TNF-alpha of GL-9 cells and TNF-alpha production in U937 cells were also examined. mAbp75R bound to U937 cells competitively with TNF-alpha and suppressed TNF-alpha production by U937, but had no effect on the growth inhibition of GL-9 human glioma cell by TNF-alpha in vitro. These findings suggest that co-administration of **TNF**-p75R **antagonist** with **TNF**-alpha may decrease the toxicity of TNF-alpha administration resulting in a better therapeutic result.

L12 ANSWER 10 OF 38 CA COPYRIGHT 1999 ACS
AN 130:94419 CA
TI Gram-negative and gram-positive bacterial products induce differential cytokine profiles in the brain: analysis using an integrative molecular-behavioral in vivo model
AU Plata-Salaman, Carlos R.; Ilyin, Sergey E.; Gayle, Dave; Flynn, Mark C.
CS Division of Molecular Biology, School of Life and Health Sciences, University of Delaware, Newark, DE, 19716-2590, USA
SO Int. J. Mol. Med. (1998), 1(2), 387-397
CODEN: IJMMFG; ISSN: 1107-3756
PB International Journal of Molecular Medicine
DT Journal
LA English
AB Bacterial-derived products [e.g., lipopolysaccharide (LPS) from Gram-neg. and muramyl dipeptide (MDP) from Gram-pos. bacteria] are proposed to play a pivotal role in the generation of **neurol**. and **neuro-inflammatory**/immunol. responses during bacterial infections of the nervous system. LPS and MDP may act through cytokines; cytokine-neuropeptide interactions may also be involved. Here, we investigated cytokine and neuropeptide mRNA profiles in specific brain regions in response to the intracerebroventricular administration of LPS and MDP. IL-1.beta. system components (ligand, signaling receptor, receptor accessory proteins, receptor **antagonist**), **TNF**-.alpha., TGF-.beta.1, glycoprotein 130 (IL-6 receptor signal transducer), OB protein (leptin) receptor, neuropeptide Y, Y5 receptor, and pro-opiomelanocortin (opioid peptide precursor) mRNAs were analyzed. The same brain region sample was assayed for all components. LPS and MDP administration induced significantly different behavioral and mol. profiles. LPS was significantly more potent than MDP in inducing anorexia and in up-regulating pro-inflammatory cytokines (IL-1.beta. and TNF-.alpha.) mRNAs in the cerebellum, hippocampus and hypothalamus; MDP was more potent in up-regulating anti-inflammatory cytokine (IL-1 receptor antagonist and TGF-.beta.1) mRNAs. LPS and MDP also modulated hypothalamic IL-1 receptor mRNA components, but did not affect any of the neuropeptide-related components examd. The results suggest that the magnitude of neurol. manifestations induced by LPS and MDP may involve the ratio between stimulatory and inhibitory cytokines, and this ratio may have implications for the neuroinflammatory/neurotoxic events assocd. with bacterial infections of the central nervous system.

L12 ANSWER 11 OF 38 CA COPYRIGHT 1999 ACS DUPLICATE 3
AN 128:242825 CA
TI Cloning and expression of murine IFN.beta. and a **TNF antagonist** for gene therapy of experimental allergic encephalomyelitis
AU Triantaphyllopoulos, K. A.; Croxford, J. L.; Baker, D.; Chernajovsky, Y.
CS Molecular Biology Laboratory, Kennedy Institute of Rheumatology, London,

W6 8LH, UK
SO Gene Ther. (1998), 5(2), 253-263
CODEN: GETHEC; ISSN: 0969-7128
PB Stockton Press
DT Journal
LA English
AB Immunomodulation of an ongoing autoimmune disease can be achieved by inhibitory cytokines or cytokine inhibitors such as **TNF antagonists**, delivered by gene therapy. The aim of this study was to design and test plasmid and retrovirus vectors expressing the mouse IFN.beta. gene and a chimeric protein contg. the extracellular domain of human p55 TNF receptor linked to a murine Ig. These vectors were transiently expressed in COS-7 cells and permanently in amphotropic packaging cell lines or ABH mouse immortalized fibroblasts. Expression levels were assessed by ELISA, Western blotting and biol. activity. In order to achieve tissue-specific expression in the CNS, the IFN.beta. gene was cloned and expressed under the control of the rat NSE promoter. We evaluated these constructs by direct intracranial injections of DNA-liposome complexes during the induction phase of exptl. allergic encephalomyelitis, a murine model of **multiple sclerosis**, with therapeutic benefit.

L12 ANSWER 12 OF 38 CA COPYRIGHT 1999 ACS DUPLICATE 4
AN 129:166 CA
TI A study of the anti-pyretic effect of quinine, an alkaloid effective against cerebral malaria, on fever induced by bacterial endotoxin and yeast in rats
AU Santos, F. A.; Rao, V. S. N.
CS Department of Physiology and Pharmacology, Health Sciences Center, Federal University of Ceara, Fortaleza, 60430-270, Brazil
SO J. Pharm. Pharmacol. (1998), 50(2), 225-230
CODEN: JPPMAB; ISSN: 0022-3573
PB Royal Pharmaceutical Society of Great Britain
DT Journal
LA English
AB The effect of quinine on fever induced by lipopolysaccharide and brewer's yeast has been investigated in rats. Oral administration of 50 or 100 mg kg-1 quinine, doses which had no effect on normothermic rats, significantly reduced lipopolysaccharide- (50 .mu.g kg-1, i.m.) and yeast-(2 g kg-1) induced fever in rats. Pentoxifylline (100 mg kg-1), a **tumor necrosis factor antagonist** also attenuated the febrile response induced by lipopolysaccharide, but not that by yeast, in a manner similar to quinine. Piroxicam (5 mg kg-1), a cyclooxygenase inhibitor suppressed both types of fever with a longer duration of action. In addn. to its anti-pyretic effect, quinine had a significant anti-inflammatory effect in the carrageenan model of acute inflammation in the hind-paw of rats. The results indicate the anti-inflammatory and anti-pyretic potential of quinine which might be important in addn. to its anti-plasmodial action in the therapy of cerebral malaria.

L12 ANSWER 13 OF 38 CA COPYRIGHT 1999 ACS
AN 130:65025 CA
TI Longitudinal study of inflammatory factors in serum, cerebrospinal fluid, and brain tissue in Alzheimer disease: interleukin-1.beta., interleukin-6, interleukin-1 receptor **antagonist**, **tumor necrosis factor**-.alpha., the soluble tumor necrosis factor receptors I and II, and .alpha.1-antichymotrypsin
AU Lanzrein, Anne-Sophie; Johnston, Carole M.; Perry, V. Hugh; Jobst, Kim A.;

King, Elizabeth M.; Smith, A. David
CS University Department of Pharmacology, Oxford, UK
SO Alzheimer Dis. Assoc. Disord. (1998), 12(3), 215-227
CODEN: ADADE2; ISSN: 0893-0341
PB Lippincott Williams & Wilkins
DT Journal
LA English
AB There is evidence consistent with the hypothesis that inflammatory and immune mechanisms are involved in the pathogenesis of Alzheimer disease (AD). We have investigated whether the levels of inflammatory assocd. proteins in serum or lumbar cerebrospinal fluid (CSF) reflect the progressive cognitive decline and brain atrophy of AD-patients. Levels of interleukin-1.beta. (IL-1.beta.), IL-1 receptor antagonist (IL-1ra), interleukin-6 (IL-6), tumor necrosis factor-.alpha. (TNF-.alpha.), the sol. TNF receptors type I and II (sTNFR I and II), and the acute phase protein .alpha.1-antichymotrypsin (.alpha.1-ACT) were detd. in paired serum and CSF samples taken yearly over a period of 2-5 yr from pathol. confirmed AD patients (n = 8) and normal controls or non-AD subjects with other CNS pathol. (n = 9). No significant differences were found between AD subjects and controls in the mean levels of the above mediators. There was also no correlation in either subject group between the levels of these inflammatory mediators in serum or CSF, and the change in cognitive status or the progression of the atrophy of the medial temporal lobe measured by x-ray computed tomog. (CT). The concns. of IL-1.beta., IL-6, and TNF-.alpha. were detd. in brain tissue specimens of five to nine different brain regions in six of the AD patients and four of the non-AD subjects. The levels of IL-1.beta. and IL-6 in the various brain regions were not significantly different in the AD and the non-AD group. However, in AD patients the level of TNF-.alpha. was significantly lower in the frontal cortex (32%, p = 0.024), the superior temporal gyrus (57%, p = 0.021), and the entorhinal cortex (49%, p = 0.009) compared with non-AD subjects. Low levels of TNF-.alpha. in the brain areas that showed neuropathol. in AD may indicate a dysregulation of the inflammatory process in AD. Despite this finding, this study does not support the use of measurements of any of the inflammatory mediators investigated here as a diagnostic parameter for AD, due the large overlap in the levels of these factors between AD patients and other subjects, and the poor relation to clin. signs of AD.

L12 ANSWER 14 OF 38 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 5
AN 1998:95186 BIOSIS
DN PREV199800095186
TI Recent bacterial and viral infection is a risk factor for cerebrovascular ischemia: Clinical and biochemical studies.
AU Grau, A. J. (1); Buggle, F.; Becher, H.; Zimmermann, E.; Spiel, M.; Fent, T.; Maiwald, M.; Werle, E.; Zorn, M.; Hengel, H.; Hacke, W.
CS (1) Dep. Neurol., Univ. Heidelberg, Im Neuenheimer Feld 400, 69120 Heidelberg Germany
SO Neurology, (Jan., 1998) Vol. 50, No. 1, pp. 196-203.
ISSN: 0028-3878.
DT Article
LA English
AB We performed a case-control study to investigate the role of recent infection as stroke risk factor and to identify pathogenetic pathways linking infection and stroke. We examined 166 consecutive patients with acute cerebrovascular ischemia and 166 patients hospitalized for nonvascular and noninflammatory **neurologic diseases**. Control subjects were individually matched to patients for sex, age, and season of admission, We assessed special biochemical parameters in subgroups of stroke patients with and without recent infection (n = 21) who were similar with respect to demographic and clinical parameters. Infection within the preceding week was a risk factor for cerebrovascular

ischemia in univariate (odds ratio (OR) 3.1; 95% confidence interval (CI), 1.57 to 6.1) and age-adjusted multiple logistic regression analysis (OR 2.9; 95% CI, 1.31 to 6.4). The OR of recent infection and age were inversely related. Both bacterial and viral infection contributed to increased risk. Infection elevated the risk for cardioembolism and tended to increase the risk for arterioarterial embolism. Stroke patients with and without preceding infection were not different with respect to factor VII and factor VIII activity, fibrin monomer, fibrin D-dimer, von Willebrand factor, C4b-binding protein, protein S, anticardiolipin antibodies, interleukin-1 receptor **antagonist**, soluble **tumor necrosis factor**-alpha receptor, interleukin-6, interleukin-8, and neopterin. In conclusion, recent infection is an independent risk factor for acute cerebrovascular ischemia. Its role appears to be more important in younger age groups. The pathogenetic linkage between infection and stroke is still insufficiently understood.

L12 ANSWER 15 OF 38 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 6
AN 1998:391502 BIOSIS
DN PREV199800391502
TI Regulation of tumour necrosis factor and interleukin-6 gene transcription by b2-adrenoceptor in the rat astrocytes.
AU Nakamura, Akio (1); Johns, Edward J.; Imaizumi, Akiro; Abe, Toshiaki; Kohsaka, Takao
CS (1) Dep. Pediatr., Teikyo Univ. Sch. Med., 2-11-1 Kaga, Itabashi-ku, Tokyo 173 Japan
SO Journal of Neuroimmunology, (Aug. 1, 1998) Vol. 88, No. 1-2, pp. 144-153.
ISSN: 0165-5728.
DT Article
LA English
AB The present study was designed to clarify the role of beta2-adrenoceptors in modulating the level of TNF and IL-6 gene transcription and their respective mRNA accumulations. Astrocytes were transfected with the 5'-flanking region of the TNF and IL-6 genes linked to a luciferase coding sequence as a reporter. The addition of isoproterenol had an inhibitory effect on the TNF and IL-6 promoter activity induced by LPS. The inhibitory effect was blocked in the presence of a beta2-adrenoceptor antagonist but not in the presence of a beta1-adrenoceptor **antagonist**. **TNF** and IL-6 mRNA and protein levels in the astrocytes were depressed by beta2-adrenoceptor activation which corresponded to changes in TNF and IL-6 promoter activity. These studies demonstrated that isoproterenol, via beta2-adrenoceptors, suppressed LPS-induced TNF and IL-6 promoter activities, mRNA accumulations, and protein levels in the astrocytes. beta2-adrenoceptor activation may be an important mechanism for regulating TNF and IL-6 expression in **brain diseases**.

L12 ANSWER 16 OF 38 CA COPYRIGHT 1999 ACS
AN 126:334212 CA
TI Cosmetic and pharmaceutical compositions containing salts of lanthanide, tin, zinc, manganese, yttrium, cobalt, strontium as substance P antagonists
IN Breton, Lionel; De Lacharriere, Olivier
PA Oreal S. A., Fr.
SO Eur. Pat. Appl., 10 pp.
CODEN: EPXXDW
DT Patent
LA French
FAN.CNT 1
PATENT NO. KIND DATE APPLICATION NO. DATE

PI	EP 770392	A2	19970502	EP 1996-402182	19961014
	EP 770392	A3	19970507		
	R: AT, BE, CH, DE, ES, FR, GB, IE, IT, LI, NL, SE				
	FR 2740335	A1	19970430	FR 1995-12658	19951026
	FR 2740335	B1	19971219		
	NO 9604517	A	19970428	NO 1996-4517	19961024
	JP 09165341	A2	19970624	JP 1996-284318	19961025
	CA 2188892	AA	19980425	CA 1996-2188892	19961025
	US 5900257	A	19990504	US 1996-738811	19961028
PRAI	FR 1995-12658	19951026			

AB The title cosmetic and pharmaceutical compns. are claimed for treatment of disorders assocd. with excess synthesis or release of substance P. A lotion contained manganese chloride 15.00, glycerol 2.00, Me paraben 0.15, perfume q.s., and water q.s. 100.00.

L12 ANSWER 17 OF 38 CA COPYRIGHT 1999 ACS
AN 126:268314 CA
TI Cosmetic and pharmaceutical compositions containing extracts of filamentous, non photosynthetic bacteria and composition containing them
IN Breton, Lionel; Aubert, Lucien; Leclaire, Jacques; Martin, Richard; De Lacharriere, Olivier
PA L'Oreal S. A., Fr.
SO Eur. Pat. Appl., 32 pp.
CODEN: EPXXDW
DT Patent
LA French
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 761204	A1	19970312	EP 1996-401781	19960813
	EP 761204	B1	19980415		
	R: DE, ES, FR, GB, IT, SE				
	FR 2738485	A1	19970314	FR 1995-10485	19950907
	FR 2738485	B1	19971114		
	FR 2746646	A1	19971003	FR 1996-3816	19960327
	FR 2746646	B1	19980515		
	FR 2746642	A1	19971003	FR 1996-3818	19960327
	FR 2746642	B1	19980515		
	WO 9709032	A1	19970313	WO 1996-FR1284	19960813
	W: BR, JP, MX, PL, RU				
	ES 2120274	T3	19981016	ES 1996-401781	19960813
	JP 10511110	T2	19981027	JP 1996-510896	19960813
	CA 2185036	AA	19970308	CA 1996-2185036	19960906
	US 5795574	A	19980818	US 1996-711109	19960909
PRAI	FR 1995-10485	19950907			
	FR 1996-3816	19960327			
	FR 1996-3818	19960327			
	WO 1996-FR1284	19960813			

AB Exts. of filamentous, non photosynthetic bacteria which are antagonist of substance P are used in cosmetic and pharmaceutical compns. for the treatment of disorders caused by excess synthesis of substance P liberation. Lyophilized ext. of Vitreoscilla filiformis was prepd. and its affinity for the NK1 receptors was studied. A cosmetic lotion contained above lyophilized ext. 0.05, glycerol 2.00, Me paraben 0.15, fragrance q.s. and water q.s. 100.00%.

L12 ANSWER 18 OF 38 DRUGU COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1997-28257 DRUGU C P
TI The first syntheses of GLA-60 positional isomers and their biological activities.
AU Shiozaki M; Arai M; Macindoe W M; Mochizuki T; Wakabayashi T; Kurakata S;

Tatsuta T; Maeda H; Nishijima M
CS Sankyo; Nat.Inst.Health-Jap.
LO Tokyo, Jap.
SO Bull.Chem.Soc.Jpn. (70, No. 5, 1149-61, 1997) 4 Fig. 16 Ref.
CODEN: BCSJA8 ISSN: 0009-2673
AV Exploratory Chemistry Research Laboratories, Sankyo Co., Ltd., Hiromachi 1-2-58, Shinagawa-ku, Tokyo 140, Japan.
LA English
DT Journal
FA AB; LA; CT
FS Literature
AB The GLA-60 positional isomer (8) and its fluorinated derivatives (14,14',20,26 and 26') were synthesized and their structures determined using IR, PMR and **MS** data. The TNFalpha inducing activities of the compounds and LPS (glycolipid), lipid A and GLA-60 (all 0.01-10 uM), were investigated using monoblastic U937 cells. The inhibitory activities of the compounds, lipid A and GLA-60 (all 0.01-10 uM) on LPS-induced TNFalpha production were also evaluated. Potent TNFalpha inducing activity was seen with LPS. TNFalpha production was also induced by lipid A in a dose dependent manner. Potent agonistic activity was exhibited by (8) whereas (26 and 26') displayed only slight agonistic activities. The difluorinated compounds (14, 14', and 20) inhibited TNFalpha production dose-dependently. Inhibition of TNFalpha production in excess of 80% was seen with (14) (10uM).

L12 ANSWER 19 OF 38 DRUGU COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1997-29369 DRUGU T P B
TI Biotechnological agents for the immunotherapy of **multiple sclerosis**. Principles, problems and perspectives.
AU Hohlfeld R
CS Univ.Munich; Max-Planck-Inst.
LO Munich, Ger.
SO Brain (120, Pt. 5, 865-916, 1997) 9 Fig. 3 Tab. 396 Ref.
CODEN: BRAIAK ISSN: 0006-8950
AV Department of Neurology, Klinikum Grosshadern, Ludwig Maximilians University, Marchioninistrasse 15, D-81366 Munich, Germany.
LA English
DT Journal
FA AB; LA; CT
FS Literature
AB Biotechnological agents for the immunotherapy of **multiple sclerosis** (**MS**) are reviewed. Based on exciting results in animal models, a number of novel immunotherapies employing biotechnological products rather than conventional immunosuppressants are being developed. The Authors first reviews some fundamental concepts of immunology (cells and molecules of the immune system; self tolerance and autoimmunity), and offers a hypothetical scenario for the immunopathogenesis of **MS**. Then follows a critical overview of various immunotherapies relying on modern technology, considering protein engineering techniques, immunological gene therapy and anti-sense oligonucleotide therapy, and principles, evidence and problems relating to various treatments for **MS**.

L12 ANSWER 20 OF 38 CA COPYRIGHT 1999 ACS DUPLICATE 7
AN 127:261519 CA
TI Interleukin-1.beta. (IL-1.beta.)-induced modulation of the hypothalamic IL-1.beta. system, tumor necrosis factor-.alpha., and transforming growth factor-.beta.1 mRNAs in obese (fa/fa) and lean (Fa/Fa) Zucker rats: implications to IL-1.beta. feedback systems and cytokine-cytokine interactions
AU Plata-Salaman, Carlos R.; Ilyin, Sergey E.

CS Division of Molecular Biology, School of Life and Health Sciences, University of Delaware, Newark, DE, USA
SO J. Neurosci. Res. (1997), 49(5), 541-550
CODEN: JNREDK; ISSN: 0360-4012
PB Wiley-Liss
DT Journal
LA English
AB Interleukin-1.beta. (IL-1.beta.) induces anorexia, fever, sleep changes, and neuroendocrine alterations when administered into the brain. Here, we investigated the regulation of the IL-1.beta. system (ligand, receptors, receptor accessory protein, and receptor **antagonist**), **tumor necrosis factor**-.alpha. (TNF-.alpha.), transforming growth factor (TGF)-.beta.1, and TGF-.alpha. mRNAs in the hypothalamus of obese (fa/fa) and lean (Fa/Fa) Zucker rats in response to the intracerebroventricular microinfusion of IL-1.beta. (8.0 ng/24 h for 72 h; a dose that yields estd. pathophysiol. concns. in the cerebrospinal fluid). IL-1.beta. increased IL-1.beta., IL-1 receptor types I and II (IL-1RI and IL-1RII), IL-1 receptor accessory protein sol. form (IL-1R AcP II), IL-1 receptor **antagonist** (IL-1Ra), **TNF**-.alpha., and TGF-.beta.1 mRNAs in the hypothalamus from obese and lean rats. IL-1.beta.-induced IL-1.beta. system and ligand (IL-1.beta., TNF-.alpha., and TGF-.beta.1) mRNA profiles were highly intercorrelated in the same samples. Levels of membrane-bound IL-1R AcP and TGF-.alpha. mRNAs did not change. Heat-inactivated IL-1.beta. had no effect. The data suggest (1) the operation of an IL-1.beta. feedback system (IL-1.beta./IL-1RI/IL-1R Acp II/ IL-1RII/IL-1Ra) and (2) potential cytokine-cytokine interactions with pos. (IL-1.beta. .tautm. TNF-.alpha.) and neg. (TGF-.beta.1 .fwdarw. IL-1.beta./TNF-.alpha.) feedback. Dysregulation of the IL-1.beta. feedback system and the TGF-.beta.1/IL-1.beta.-TNF-.alpha. balance may have implications for **neurol. disorders** assocd. with high levels of IL-1.beta. in the brain.

L12 ANSWER 21 OF 38 CA COPYRIGHT 1999 ACS DUPLICATE 8
AN 127:233468 CA
TI Cerebrospinal fluid interleukin-1 receptor **antagonist** and **tumor necrosis factor**-.alpha. following subarachnoid hemorrhage
AU Mathiesen, Tiit; Edner, Goran; Ulfarsson, Elfar; Andersson, Birger
CS Department of Neurosurgery, Karolinska Hospital, Stockholm, Swed.
SO J. Neurosurg. (1997), 87(2), 215-220
CODEN: JONSAC; ISSN: 0022-3085
PB American Association of Neurological Surgeons
DT Journal
LA English
AB Subarachnoid hemorrhage (SAH) causes an inflammatory reaction and may lead to ischemic brain damage. Exptl. ischemia has been shown to be connected with the alarm-reaction cytokines interleukin-1 receptor antagonist (IL-1Ra) and tumor necrosis factor-.alpha. (TNF.alpha.). Increased levels of these cytokines, however, have not been detected thus far in patients following an SAH event. For this reason daily cerebrospinal fluid (CSF) samples were collected from 22 consecutively enrolled patients with SAH and from 10 non-SAH patients (controls). The CSF samples were studied using immunoassays for IL-1Ra and TNF.alpha. to investigate whether an SAH caused increased cytokine levels. The mean IL-1Ra levels were significantly higher in patients with SAH who were in poor clin. condition on admission than in those who were in good condition (318 pg/mL vs. 82 pg/mL). The IL-1Ra levels increased during delayed ischemic episodes and after surgery in patients who were in poor clin. condition. Significant

increases in IL-1Ra and TNF.alpha. were detected during Days 4 through 10 in patients suffering from SAH who eventually had a poor outcome. Patients with good outcomes and control patients had low levels of these cytokines. The levels of IL-1Ra increased after surgery in patients with Hunt and Hess Grades III through V, but not in those with Grade I or II. This finding indicates that patients in poor clin. condition have a labile biochem. state in the brain that is reflected in increased cytokine levels following the surgical trauma. Both IL-1Ra and TNF.alpha. are known to induce fever, malaise, leukocytosis, and nitric oxide synthesis and to mediate ischemic and **traumatic brain injuries** . The present study shows that levels of these cytokines increase after SAH occurs and that high cytokine levels correlate with brain damage. It is therefore likely that fever, leukocytosis, and nitric oxide synthesis are also mediated by IL-1 in patients suffering from SAH and it is probable that the inflammatory mediators contribute to brain damage.

L12 ANSWER 22 OF 38 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 9
AN 1997:442170 BIOSIS
DN PREV199799741373
TI HIV-1 envelope glycoprotein 120 regulates brain IL-1-beta system and TNF-alpha mRNAs in vivo.
AU Ilyin, Sergey E.; Plata-Salaman, Carlos R. (1)
CS (1) Div. Molecular Biol., Sch. Life Health Sci., Univ. Delaware, Newark, DE 19716-2590 USA
SO Brain Research Bulletin, (1997) Vol. 44, No. 1, pp. 67-73.
ISSN: 0361-9230.
DT Article
LA English
AB Human immunodeficiency virus type I (HIV-1)-derived envelope glycoprotein 120 (gp120) is proposed to play an important role in HIV-1 neuropathology. Gp120 may act through mediators including proinflammatory cytokines. Here, we investigated the regulation of the IL-1-beta system (IL-1-beta, IL-1 receptor type I (IL-1RI), IL-1 receptor **antagonist** (IL-1Ra)), **TNF**-alpha and TGF-alpha mRNAs in the rat central nervous system (CNS) in response to the constant intracerebroventricular (ICV) microinfusion of HIV-1 gp120 for 72 h and 144 h. The results show that gp120: (1) increased IL-1-beta and IL-1 Ra mRNAs levels in the same samples from the cerebellum, hypothalamus and midbrain, with the largest increase in the hypothalamus; (2) induced profiles of IL-1-beta mRNA and IL-1 Ra mRNA that were highly intercorrelated; (3) increased the hypothalamic TNF-alpha mRNA levels; and (4) did not affect the IL-1RI mRNA and TGF-alpha mRNA levels in any brain region. A dysregulation in the IL-1-beta/IL-1Ra CNS balance and a mutual induction and synergistic activity of IL-1-beta and TNF-alpha could result in a deleterious amplification cycle of cellular activation and cytotoxicity with implications to HIV-1-associated encephalitis, encephalopathy, and neurological manifestations.

L12 ANSWER 23 OF 38 CA COPYRIGHT 1999 ACS
AN 125:212675 CA
TI 1,4,5-Trisubstituted imidazoles useful as cytokine suppressors
IN Adams, Jerry Leroy; Gallagher, Timothy F.; Garigipati, Ravi Shanker; Boehm, Jeffrey Charles; Sisko, Joseph; Peng, Zhi-Qiang; Lee, John Cheung-Lun
PA Smithkline Beecham Corporation, USA
SO PCT Int. Appl., 82 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 5

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9621452	A1	19960718	WO 1996-US546	19960111
	W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, PT, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, US, US, UZ, VN				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5593992	A	19970114	US 1995-472366	19950607
	ZA 9600094	A	19960724	ZA 1996-94	19960108
	AU 9646572	A1	19960731	AU 1996-46572	19960111
	AU 705207	B2	19990520		
	BR 9606904	A	19971021	BR 1996-6904	19960111
	EP 809499	A1	19971203	EP 1996-902151	19960111
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI				
	JP 10512555	T2	19981202	JP 1996-521862	19960111
	NO 9703167	A	19970908	NO 1997-3167	19970708
	FI 9702901	A	19970908	FI 1997-2901	19970708

PRAI US 1995-369964 19950109
US 1995-472366 19950607
US 1993-92733 19930716
WO 1996-US546 19960111

OS CASREACT 125:212675; MARPAT 125:212675

AB Imidazole derivs. I [R1 = (substituted) 4-pyridyl, pyrimidinyl, quinolyl, isoquinolyl, quinazolin-4-yl, 1-imidazolyl, 1-benzimidazolyl; R2 = (substituted) C1-10 alkyl, C2-10 alkenyl or alkynyl, N3, cycloalkyl, heterocyclyl, etc.; R4 = (substituted) Ph, 1- or 2-naphthyl, heteroaryl] are prepd. which inhibit mitogen-activated protein kinase and the secretion of interleukin 1 and tumor necrosis factor and are useful in treatment of cytokine-mediated inflammatory diseases. Thus, 1-[3-(4-morpholinyl)propyl]-4-(4-fluorophenyl)-5-(4-pyridyl)imidazole (II)
inhibited lipopolysaccharide-induced prostaglandin endoperoxide synthase-2
expression in human monocytes with a potency similar to that of dexamethasone. II was prepd. by condensation of pyridine-4-carboxaldehyde
with 4-(3-aminopropyl)morpholine and reaction of the product with 4-fluorophenyl-tolylthiomethylisocyanide (prepd. from p-fluorobenzaldehyde, thiocresol, and HCONH2).

L12 ANSWER 24 OF 38 DRUGU COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1996-26741 DRUGU C P
TI Carbocyclic nucleosides as inhibitors of human tumor necrosis factor-alpha production: effects of the stereoisomers of (3-hydroxycyclopentyl)adenines.
AU Borcherding D R; Peet N P; Munson H R; Zhang H; Hoffman P F; Bowlin T L; Edwards C K III
CS Hoechst-Marion-Roussel
LO Cincinnati, Ohio, USA
SO J.Med.Chem. (39, No. 13, 2615-20, 1996) 1 Tab. 24 Ref.
CODEN: JMCMAR ISSN: 0022-2623
AV Discovery Chemistry, 2110 East Galbraith Rd., P.O. Box 156300, Cincinnati, OH 45215-6300, U.S.A.
LA English
DT Journal
FA AB; LA; CT
FS Literature
AB A series of 4 structurally-related carbocyclic nucleosides was prepared and evaluated as inhibitors of tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), and IL-6 production from human primary macrophages; the compounds had little effect on IL-1beta and IL-6 production. MDL-201484 (10a) was a potent inhibitor of TNF-alpha

production. Given i.p. (10a) protected against lethal challenges of lipopolysaccharide (LPS) and D-galactosamine (D-Gal) in a septic shock model in mice.

L12 ANSWER 25 OF 38 CA COPYRIGHT 1999 ACS DUPLICATE 10
AN 125:273313 CA
TI In vivo regulation of the IL-1.beta. system (ligand, receptors I and II, receptor accessory protein, and receptor **antagonist**) and **TNF**-.alpha. mRNAs in specific brain regions
AU Ilyin, Sergey E.; Plata-Salaman, Carlos R.
CS Division of Molecular Biology, Univ. of Delaware, Newark, DE, 19716-2590, USA
SO Biochem. Biophys. Res. Commun. (1996), 227(3), 861-867
CODEN: BBRCA9; ISSN: 0006-291X
DT Journal
LA English
AB Interleukin-1.beta. (IL-1.beta.) acts directly in the central nervous system (CNS). Here, using a novel behavioral-mol. approach, we report the regulation of the complete IL-1.beta. system (ligand, receptors, receptor accessory protein, and receptor **antagonist**) and **TNF**-.alpha. mRNAs in the CNS is response to the chronic intracerebroventricular microinfusion of IL-1.beta.. IL-1.beta. increased the IL-1.beta. system and TNF-.alpha. mRNAs in the cerebellum and parieto-frontal cortex. IL-1.beta.-induced profiles of IL-1.beta., IL-1 receptor type I and II (IL-1RI and IL-1RII), and IL-1 receptor antagonist (IL-1Ra) mRNAs were highly intercorrelated in the same samples. The data suggest the operation of an IL-1.beta. feedback system (IL-1.beta./IL-1RI/IL-1RII/IL-1Ra) within a brain region. The fine regulation of the CNS IL-1.beta. system may depend on a balance between the ligand (IL-1.beta.) action on the IL-1RI and the induction of inhibitory mechanisms (IL-1RII and IL-1Ra). This may have implications regarding **neurol. diseases** assocd. with high levels of IL-1.beta. in the brain.

L12 ANSWER 26 OF 38 CA COPYRIGHT 1999 ACS
AN 124:194348 CA
TI PEGylation reagents and biologically active compounds formed therewith
IN Kohno, Tadahiko; Kachensky, Dave; Harris, Milton
PA USA
SO PCT Int. Appl., 66 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9534326	A1	19951221	WO 1995-US7555	19950614
W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT				
RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2191971	AA	19951221	CA 1995-2191971	19950614
AU 9528286	A1	19960105	AU 1995-28286	19950614
EP 758906	A1	19970226	EP 1995-923865	19950614
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
BR 9507999	A	19970812	BR 1995-7999	19950614
CN 1158089	A	19970827	CN 1995-194463	19950614
HU 77529	A2	19980528	HU 1996-3442	19950614
FI 9604985	A	19961216	FI 1996-4985	19961212

NO 9605342 A 19970214 NO 1996-5342 19961212
PRAI US 1994-259413 19940614
WO 1995-US7555 19950614
AB Biol. active conjugates are disclosed which are formed by reaction of a thiol moiety of a biol. active mol. with a non-peptidic polymer having an active sulfone moiety. Also disclosed are compds. having the formula R1-R2 wherein at least one of R1 and R2 is a biol. active mol. having a reactive thiol moiety which forms a covalent bond with X, a Michael acceptor-activated non-peptidic polymer. Further disclosed are methods of making the conjugates and compds. of the present invention as well as pharmaceutical compns. contg. them. In addn., activated polymers suitable for attachment to a variety of mols. and surfaces are disclosed. Among the reagents synthesized is e.g. a vinyl sulfone NHS-ester heterobifunctional PEG(3400) reagent. Also described are prepn. of conjugates of PEG reagents with IL-1ra (interleukin-1 receptor **antagonist**) and with **TNF** binding protein c105 mutein. A TNFbp c105 dumbell (prepd. with PEG-bis-vinyl sulfone) inhibited exptl. allergic encephalomyelitis, reduced central **nervous** system **inflammation**, and protected against endotoxin lethality.

L12 ANSWER 27 OF 38 CA COPYRIGHT 1999 ACS DUPLICATE 11
AN 123:167221 CA
TI Cytokine regulation of astrocyte function: in-vitro studies using cells from the human brain
AU Aloisi, Francesca; Borsellino, Giovanna; Care, Alessandra; Testa, Ugo; Gallo, Paolo; Russo, Giovanni; Peschle, Cesare; Levi, Giulio
CS Department of Organ and System Pathophysiology, Istituto Superiore di Sanita, Rome, Italy
SO Int. J. Dev. Neurosci. (1995), Volume Date 1995, 13(3/4), 265-74
CODEN: IJDND6; ISSN: 0736-5748
DT Journal
LA English
AB Participation of astrocytes in central nervous system pathophysiol. is likely to involve cytokines, both as stimulators and mediators of astrocyte function. We have used highly enriched human astrocyte cultures as an exptl. tool to investigate the influence of cytokines on adhesion mol. expression and synthesis of mediators that are probably important in immune and inflammatory reactions involving the nervous system and in cerebral tissue repair. The response of astrocytes to interferon-.gamma. mainly resulted in increased expression of major histocompatibility complex antigens and co-stimulatory mols. (intercellular adhesion mol.-1, LFA-1.alpha.) which mediate astrocyte-T-cell interactions. Another co-stimulatory mol., B7, was neither expressed nor inducible by IFN-.gamma. and other cytokines. TNF-.alpha. and IL-1.beta. were more efficient in stimulating synthesis of immunoregulatory and proinflammatory cytokines (IL-6, IL-8 and colony-stimulating factors), cytokine **antagonists** (**TNF**-.alpha. sol. receptors), or cytokines with a possible neuroprotective role (leukemia inhibitory factor); they also increased expression of some co-stimulatory mols. (intercellular adhesion mol.-1 and vascular cell adhesion mol.-1). Transforming growth factor-.beta.1 was a strong inducer of leukemia inhibitory factor, but did not affect either major histocompatibility complex/co-stimulatory mol. expression or cytokine synthesis. Thus, different cytokines activate distinct functional programs in astrocytes, which may play a specific role in different **brain diseases** or at different stages of the same disease. The response of human astrocytes to cytokines (in particular the inducible synthesis of certain cytokines) varied greatly depending on the presence or absence of neurons in the culture system. This finding suggests that neuronal-glial interactions may be implicated

in detg. the activation threshold of astrocytes to inflammatory cytokines.

L12 ANSWER 28 OF 38 CA COPYRIGHT 1999 ACS
AN 124:200069 CA
TI Expression of IL-1ra and TNF-.alpha. genes in lymphocytes of patients suffering graft vs. host disease (GVHD)
AU Dong, Xuebing; Wang, Shengwu; Lu, Daopei
CS Inst. Hematology, Beijing Med. Univ., Beijing, 100029, Peop. Rep. China
SO Beijing Yike Daxue Xuebao (1995), 27(3), 215
CODEN: BYDXEV; ISSN: 1000-1530
DT Journal
LA Chinese
AB Eight leukemia patients suffering from GVHD and 8 patients without GVHD, after receiving heterogenetic bone marrow transplantation, were subjected to IL-1ra and TNF-.alpha. gene expression examn. by blot hybridization and chemiluminescence autoradiog. The results showed 6/8 (75%) IL-1ra pos. and 8/8 (100%) TNF-.alpha. pos. in the GVHD group, and 1/8 (12.5%) IL-1ra pos. and 1/8 (12.5%) TNF-.alpha. pos. in the non-GVHD group. The results suggest that the prodn. and activation of a large amt. of monocytic cytokines resulted in "cytokine explosion" and thus induced a series of immunol. injurious processes and various pathol. **lesions** of GVHD, which might be depressed by antibodies against cytokines and/or cytokine receptor antagonists.

L12 ANSWER 29 OF 38 DRUGU COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1995-13785 DRUGU P M
TI Effect of interleukin-1 receptor **antagonist** and soluble **tumor necrosis factor** receptor in animal models of infection.
AU Paris M M; Friedland I R; Ehrett S; Hickey S M; Olsen K D; Hansen E; Thonar E J M A; McCracken G H
CS Univ.Texas
LO Dallas, Tex.; Chicago, Ill., USA
SO J.Infect.Dis. (171, No. 1, 161-19, 1995) 3 Fig. 5 Tab. 44 Ref.
CODEN: JIDIAQ ISSN: 0022-1899
AV Department of Pediatrics, Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9065, U.S.A. (G.H.M.).
LA English
DT Journal
FA AB; LA; CT
FS Literature
AB Soluble human tumor necrosis factor receptor (sTNFR, Immunex) and human interleukin-1 receptor antagonist (IL-1RA), given intraarticularly or intracisternally, reduced the inflammatory response induced in rabbits by intraarticular or intracisternal inoculation of rabbit tumor necrosis factor-alpha (TNF-a, Dainippon) and rabbit recombinant interleukin-1-beta (rIL-1b). IL-1RA and sTNFR had no consistent modulatory effect on the inflammatory response induced by intraarticular or intracisternal inoculation with Haemophilus influenzae type b (Hib) or Hib lipooligosaccharide (LOS) in models of arthritis or meningitis, respectively. When sTNFR was given +/- IL-1RA in rabbits inoculated with Hib LOS, there was inconsistent modulation of meningeal **inflammation**, and **brain** edema was not reduced.

L12 ANSWER 30 OF 38 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 95324612 EMBASE
DN 1995324612
TI G 619, a dual thromboxane synthase inhibitor and thromboxane A2 receptor **antagonist**, inhibits **tumor necrosis factor**-.alpha. biosynthesis.

AU Altavilla D.; Squadrito F.; Canale P.; Ioculano M.; Squadrito G.; Campo G.M.; Serrano M.; Sardella A.; Urna G.; Spignoli G.; Caputi A.P.
CS Institute of Pharmacology, School of Medicine, University of Messina, Piazza XX Settembre 4,98122 Messina, Italy
SO European Journal of Pharmacology, (1995) 286/1 (31-39).
ISSN: 0014-2999 CODEN: EJPHAZ
CY Netherlands
DT Journal; Article
FS 004 Microbiology
005 General Pathology and Pathological Anatomy
018 Cardiovascular Diseases and Cardiovascular Surgery
029 Clinical Biochemistry
048 Gastroenterology
030 Pharmacology
037 Drug Literature Index
LA English
SL English
AB G 619 is 3-carbamyl-(3'-picolyl)-4-methoxy-1-benzamide. The compound is structurally related to picotamide, a previously reported dual thromboxane synthase inhibitor/thromboxane A2 receptor antagonist, which displays inhibitory activity on tumor necrosis factor-.alpha.. The aim of the present work was to study the effect of G 619 on tumor necrosis factor-.alpha. synthesis both in vivo and in vitro. Salmonella enteritidis lipopolysaccharide was used to induce tumor necrosis factor-.alpha. production. Septic shock was produced in male rats by a single intravenous (i.v.) injection of 20 mg/kg (LD90) of Salmonella enteritidis lipopolysaccharide. Rats were pretreated with G 619 (50 mg/kg, i.v.) or vehicle (1 ml/kg, i.v.) 1 h before endotoxin challenge. Salmonella enteritidis lipopolysaccharide administration dramatically reduced survival rate (0%, 72 h after endotoxin administration), reduced mean arterial blood pressure, increased plasma levels of thromboxane B2 and 6-keto-prostaglandin F(1.alpha.) and enhanced serum levels of tumor necrosis factor. Furthermore, endotoxic shock produced characteristic gastric damage, consisting of haemorrhagic infiltrates. Pretreatment with G 619 in vivo significantly protected against Salmonella enteritidis lipopolysaccharide-induced lethality (80% survival rate and 60% survival rate 24 h and 72 h after Salmonella enteritidis lipopolysaccharide injection, respectively), reduced hypotension, decreased plasma thromboxane B2 and serum tumor necrosis factor-.alpha. levels and enhanced blood levels of 6-keto-prostaglandin F(1.alpha.). In rat peritoneal macrophages, G 619 in vitro (25, 50 and 100 .mu.M) significantly blunted ($P < 0.001$) Salmonella enteritidis lipopolysaccharide-stimulated production of tumor necrosis factor-.alpha., whereas it increased 6-keto-prostaglandin F(1.alpha.) and cyclic AMP levels. The present data indicate that G 619 may be useful during disease states characterized by elevated tumor necrosis factor-.alpha. levels.

L12 ANSWER 31 OF 38 DRUGU COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1995-36639 DRUGU P S
TI **Tumor necrosis factor** alpha receptor-**antagonists** protect against indomethacin-induced gastric mucosal injury in rats.
AU Santucci L; Fiorucci S; Brunori P M; Di Matteo F M; Chiorean M; Morelli A
CS Univ.Perugia
LO Perugia, It.
SO Gastroenterology (108, No. 4, Suppl., A209, 1995)
CODEN: GASTAB ISSN: 0016-5085
AV Gastroenterology Department, University of Perugia, Italy.
LA English
DT Journal

FA AB; LA; CT
FS Literature
AB Margination of neutrophils (PMN) into the gastric microcirculation is a critical and early event in the pathogenesis of gastric mucosal injury induced by NSAIDs. Tumor necrosis factor alpha (TNF) is a proinflammatory cytokine that causes PMN margination by upregulating the expression of adhesion molecules on both PMN and endothelial cells. The 1st step in TNF action is its binding to 2 specific cell-surface receptors, of 55 kdaltons (p55 or TNF-R1) and 75 kdaltons (p75 or TNF-R2), respectively. In this study, in-vivo treatment with monoclonal antibodies directed against TNF-R1 (Htr-9) or TNF-R2 (Utr-1) receptor protect rat gastric mucosa against indomethacin-induced damage showing that indomethacin-induced gastric mucosal damage is a TNF-mediated process. (conference abstract).

L12 ANSWER 32 OF 38 DRUGU COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1994-43323 DRUGU T S
TI Effects of thalidomide on progressive weight loss in patients with late stage of HIV disease. Preliminary results of a clinical trial.
AU Reyes Teran G; Sierra Madero J G; Martinez del Cerro V; Munoz T; Arroyo H; Pasquetti A
CS Nat.Inst.Nutr.Mexico
LO Mexico City, Mexico
SO Clin.Infect.Dis. (19, No. 3, 612, 1994) ISSN: 1058-4838
AV National Institute of Nutrition, Mexico City, Mexico. (8 authors).
LA English
DT Journal
FA AB; LA; CT
FS Literature
AB The effects of p.o. thalidomide (THAL) were studied on progressive weight loss (PWL) in 23 patients with late stage HIV disease in a randomized, double-blind, placebo-controlled clinical trial. Its effect on circulating CD4+ T-cells, HIV viral burden in peripheral blood mononuclear cells (PBMC), and plasma TNFalpha levels was also investigated. It was concluded that THAL significantly increased body weight in patients with late stage of AIDS. The Authors results suggest that THAL, at the dosage used in this study, had no in-vivo effect on HIV viral burden, and had no important toxicity. Mild and transient somnolence and erythematous macular **lesions** were only observed in the THAL group. (conference abstract).

L12 ANSWER 33 OF 38 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 12
AN 1995:64195 BIOSIS
DN PREV199598078495
TI Molecular aspects of granulomatous inflammation.
AU Kunkel, S. L. (1); Strieter, R. M.; Lukacs, W. (1); Chensue, S. W. (1)
CS (1) Dep. Pathol., Univ. Michigan Sch., Ann Arbor, MI USA
SO EOS-Rivista di Immunologia ed Immunofarmacologia, (1994) Vol. 14, No. 2, pp. 71-77.
ISSN: 0392-6699.
DT Article
LA English
SL English; Italian
AB Although granulomatous diseases have been historically recognized as important pathological entities, the specific mechanisms that control the initiation and maintenance of the inflammatory response is not clear A number of studies have clearly demonstrated that certain cytokines, including IL-1 and TNF, play a prominent role in the development of a granulomatous response. These early response cytokines play key roles during both the initiation and maintenance of the **lesion** via the generation of cytokine networks. The establishment of these communication circuits between inflammatory cells and nonimmune resident tissue cells is

an important cells is an important consequence for the successful evolution and repair of the chronic inflammatory response. For example, both IL-1 and TNF can serve as activating cytokines and stimulate stromal, epithelial, and endothelial cells to become participants in the developing chronic immune response. Important consequences of IL-1 and TNF stimulation is the expression of key mediators needed to elicit the correct leukocyte population to the inflamed area. Both adhesion molecules and chemokines are generated under the 'guidance' of IL-1 and TNF, which are needed to initiate and sustain the chronic inflammatory **lesion**. The recruitment phase of granulomatous inflammation has long been an enigma, as the mechanisms which insures the timely arrival of mononuclear cells to the specific site of **lesion** development are not well understood. However, recent insights into leukocyte recruitment have shown that C-C chemokines are prime mediators of specific leukocyte recruitment.

The generation of the appropriate chemokine(s) are dependent upon the 'cytokine cocktail' which is expressed as the antigen dependent **lesion** evolves. This cytokine phenotype is dictated in part by the inciting antigen and the involvement of Th1- or Th2-type lymphocytes. Thus, lymphocyte products, such as IL-4, IL-10, gamma IFN, and IL-2, are equally as important as macrophage-derived IL-1 and TNF. The importance of the latter two early response cytokines is underscored by the identification of endogenous regulators of IL-1 and TNF activity. Interleukin-1 receptor **antagonist** and soluble **TNF** receptors are apparently generated coincidentally with IL-1 and TNF in order to maintain a balance of biologically active mediators, The balance of inflammatory mediators is important in that it insures that the immune response can progress with a minimum of normal tissue injury. While our knowledge of the mechanisms behind initiation and maintenance of granulomatous inflammation is expanding, our applied knowledge for the treatment of granulomatous diseases is delinquent. However, as the scientific community makes inroads to fully understand the specific mechanisms responsible for these chronic states, more specific and efficacious treatment protocols will be developed and utilized.

L12 ANSWER 34 OF 38 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 94375028 EMBASE
DN 1994375028
TI Detection of transforming growth factor beta1 mRNA in cerebrospinal fluid cells of patients with meningitis by non-radioactive in situ hybridization.
AU Ossege L.M.; Voss B.; Wiethege T.; Sindern E.; Malin J.-P.
CS Department of Neurology, Ruhr-University Bochum, BG Klinikum Bergmannsheil, Gilsingstrasse 14,D-44789 Bochum, Germany
SO Journal of Neurology, (1994) 242/1 (14-19).
ISSN: 0340-5354 CODEN: JNRYA
CY Germany
DT Journal; Article
FS 005 General Pathology and Pathological Anatomy
008 Neurology and Neurosurgery
LA English
SL English
AB Meningitis is a serious disease mostly caused by viral or bacterial infections. In complicated cases it may lead to brain damage and death. The infection and cell damage result in a cellular and immunological response. Following this, a high secretion of cytokines can be expected. Cytokines, especially tumour necrosis factor alpha (TNF-alpha) and interleukin-1 (IL-1), promote the inflammatory reactions in the subarachnoid space. Transforming growth factor beta1 (TGF-beta1) has **antagonistic** effects on **TNF**-alpha and IL-1-mediated

processes. Therefore, it suppresses inflammatory reactions. To observe the expression of TGF-beta1 in transcellular signalling in the inflammatory processes of meningitis, we investigated TGF-beta1 mRNA in cells in the cerebrospinal fluid of three patients with meningitis by non-radioactive in situ hybridization. All patients fulfilled the usual clinical criteria of meningitis. In one case Neisseria menigitidis could be identified as the pathogenic agent. In the remainder, no agent could be isolated. In all cytological preparations of the cerebrospinal fluid of these patients a high level of TGF-beta1 mRNA was detectable in the cell populations. It was possible to distinguish between the different cell types of the cerebrospinal fluid and to attach the mRNA expression to them. On the one hand, this makes it possible to investigate pathogenesis and defence mechanisms in bacterial and aseptic meningitis on a cellular level; on the other hand, it may open new perspectives in the control of disease development, prognosis, diagnosis and supporting therapy.

L12 ANSWER 35 OF 38 DRUGU COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1993-03263 DRUGU T
TI New Perspectives in Immunotherapy: Adhesion molecules, Super-antigens, Heat-Shock Proteins and Cytokine Antagonists.
AU Gause A; Sahin U; Pfreundschuh
LO Homburg, Germany,West
SO Dtsch.Med.Wochenschr. (117, No. 46, 1764-73, 1992) 3 Fig. 2 Tab. 59 Ref.
CODEN: DMWOAX ISSN: 0012-0472
AV Abteilung Innere Medizin I, Medizinische Klinik und Poliklinik der Universitaet des Saarlandes, W-6650 Homburg, Germany.
LA German
DT Journal
FA AB; LA; CT
FS Literature
AB This review discusses recent knowledge concerning the immune response, its pathophysiological importance in certain diseases and possible therapeutic uses of adhesion molecules (AM), Major Histocompatibility Complex (MHC) Molecules, T cell receptor/CD3 complex, superantigens, heat shock proteins (HSP), cytokines and their antagonists. Antibodies or antagonists to AM may prove useful in AIDS, rheumatoid arthritis (RA), **multiple sclerosis** (**MS**) and after MI. Monoclonal antibodies (MAB) to CD3 are already licensed for GVHD and possible uses of T cell vaccines are under study. Superantigens coupled to tumor-associated MAB may prove useful in tumor therapy. Indications for cytokines e.g. interleukin (IL), IFN and **TNF** and their **antagonists** include malignant diseases, allergies and septic shock.

L12 ANSWER 36 OF 38 MEDLINE
AN 93159169 MEDLINE
DN 93159169
TI Peptides and cytokines.
AU Schroder J M
CS Department of Dermatology, University of Kiel, Federal Republic of Germany.
SO ARCHIVES OF DERMATOLOGICAL RESEARCH, (1992) 284 Suppl 1 S22-6. Ref: 35
Journal code: 6X7. ISSN: 0340-3696.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LA English
FS Priority Journals
EM 199305
AB In the last decade a number of proteinaceous inflammatory mediators have

been structurally characterized. Two of these mediators, tumor necrosis factor alpha (TNF alpha) and Interleukin 1 alpha and beta (IL-1), have pleiotropic properties. Both cytokines are now known to be potent inducers of a number of cell-selective chemotactic cytokines, which belong to a novel superfamily of structurally related low-molecular-weight proteins. One of the most prominent members is termed "IL-8" and represents a neutrophil-selective attractant, whereas another one called "monocyte chemotactic protein 1 (MCP-1)" is a monocyte-selective chemotaxin. Other members seem to be selective chemotaxins for other leukocyte types and subsets. These chemotactic cytokines are produced by a variety of different cells under appropriate stimulation conditions. Large amounts of IL-8 have been detected in scales of psoriatic **lesions** and may be of importance in explaining predominant neutrophil infiltration in psoriatic **lesions**. Regulation of gene expression and/or release of these chemotactic cytokines may occur by IL-1 receptor **antagonists** or soluble **TNF**-alpha-receptors. So far, natural antagonists to these chemotactic cytokines have not been described; however, pharmacological inhibition of its gene expression and/or release is possible.

L12 ANSWER 37 OF 38 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 92370962 EMBASE
DN 1992370962
TI Peptides and cytokines.
AU Schroder J.-M.
CS Department of Dermatology, University of Kiel, Schittenhelmstrasse 7,W-2300 Kiel, Germany
SO Archives of Dermatological Research, (1992) 284/SUPPL. 1 (S22-S26). ISSN: 0340-3696 CODEN: ADMFAU
CY Germany
DT Journal; Conference Article
FS 005 General Pathology and Pathological Anatomy
013 Dermatology and Venereology
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LA English
SL English
AB In the last decade a number of proteinaceous inflammatory mediators have been structurally characterized. Two of these mediators, tumor necrosis factor .alpha. (TNF.alpha.) and Interleukin 1.alpha. and .beta. (IL-1), have pleiotropic properties. Both cytokines are now known to be potent inducers of a number of cell-selective chemotactic cytokines, which belong to a novel superfamily of structurally related low-molecular-weight proteins. One of the most prominent members is termed 'IL-8' and represents a neutrophil-selective attractant, whereas another one called 'monocyte chemotactic protein 1 (MCP-1)' is a monocyte-selective chemotaxin. Other members seem to be selective chemotaxins for other leukocyte types and subsets. These chemotactic cytokines are produced by a variety of different cells under appropriate stimulation conditions. Large amounts of IL-8 have been detected in scales of psoriatic **lesions** and may be of importance in explaining predominant neutrophil infiltration in psoriatic **lesions**. Regulation of gene expression and/or release of these chemotactic cytokines may occur by IL-1 receptor **antagonists** or soluble **TNF**-.alpha.-receptors. So far, natural antagonists to these chemotactic cytokines have not been described; however, pharmacological inhibition of its gene expression and/or release is possible.

L12 ANSWER 38 OF 38 DRUGU COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1989-19228 DRUGU P T S
TI Tumour Necrosis Factor.
AU Balkwill F R
LO London, United Kingdom
SO Br.Med.Bull. (45, No. 2, 389-400, 1989) 39 Ref.
CODEN: BMBUAQ ISSN: 0007-1420
AV Imperial Cancer Research Fund, Lincoln's Inn Fields, London, England.
LA English
DT Journal
FA AB; LA; CT
FS Literature
AB The pathophysiological actions of tumor-necrosis-factor (TNF) is reviewed. The relationship between TNF and lymphotoxin (LT) is discussed. The cytotoxic and anticancer activities of TNF are reviewed. TNF cell surface receptors are discussed. The role of TNF in disease and immune cell killing is discussed. The effects of TNF on cells involved in immunity and inflammation and its effects on bone and cartilage are described. Side-effects of TNF when administered directly into human tumors include flu-like symptoms, fever, rigors and headaches.

=> d his

(FILE 'HOME' ENTERED AT 17:38:04 ON 15 SEP 1999)

FILE 'CA, BIOSIS, MEDLINE, DRUGU, EMBASE' ENTERED AT 17:38:47 ON 15 SEP 1999

L1 864 S (TNF OR TUMOR NECROSIS FACTOR?)(2A)ANTAGONIST?
L2 551832 S (NEURO? OR NERV? OR SPINAL CORD OR BRAIN)(2A)(DISEAS? OR DISO
L3 2764507 S HERNIATED DISC? OR CARPAL TUNNEL SYNDROME OR PITUITARY ADENOM
L4 829 S L1 AND (L2 OR L3)
L5 381 S L1(10A)(L2 OR L3)
L6 224 DUP REM L5 (157 DUPLICATES REMOVED)
L7 190 S L6 NOT PY>1998
L8 213100 S HIS
L9 1188640 S HERNIATED DISC? OR CARPAL TUNNEL SYNDROME OR PITUITARY ADENOM
L10 3 S L1(10A)(L2 OR L9)
L11 64 S L1 AND (L2 OR L9)
L12 38 DUP REM L11 (26 DUPLICATES REMOVED)

```
=> e etanercept/cn

E1            1     ETANAUTINE/CN
E2            1     ETANDAN/CN
E3            1 --> ETANERCEPT/CN
E4            1     ETANIDAZOLE/CN
E5            1     ETANOR/CN
E6            1     ETANTEROL/CN
E7            1     ETAP/CN
E8            1     ETAPAK/CN
E9            1     ETAPERAZIN/CN
E10           1     ETAPERAZINE/CN
E11           1     ETAPHEN/CN
E12           1     ETAPHOS/CN

=> s e3

L1            1 ETANERCEPT/CN

=> d


L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 1999 ACS
RN   185243-69-0  REGISTRY
CN   1-235-Tumor necrosis factor receptor (human) fusion protein with
     236-467-immunoglobulin G1 (human .gamma.1-chain Fc fragment) (9CI)  (CA
     INDEX NAME)
OTHER NAMES:
CN   Embrel
CN   Enbrel
CN   Etanercept
CN   rhu TNFR:Fc
FS   PROTEIN SEQUENCE
DR   200013-86-1
MF   Unspecified
CI   MAN
SR   US Adopted Names Council
LC   STN Files:   ADISINSIGHT, BIOSIS, CA, CAPLUS, IPA, MRCK*, PHAR, TOXLINE,
       TOXLIT
         (*File contains numerically searchable property data)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               10 REFERENCES IN FILE CA (1967 TO DATE)
               11 REFERENCES IN FILE CAPLUS (1967 TO DATE)


=> e infliximab/cn

E1            1     INFLEXUSIN/CN
E2            1     INFLEXUSIN B/CN
E3            1 --> INFLIXIMAB/CN
E4            1     INFLUINA/CN
E5            1     INFLUMIN/CN
E6            1     INFO 1/CN
E7            1     INFO 2/CN
```

```
E8            1      INFO 5/CN
E9            1      INFO 531/CN
E10           1      INFOLITE ER 51/CN
E11           1      INFONUTROL/CN
E12           1      INFORM 6350M/CN

=> s e3

L2            1 INFLIXIMAB/CN

=> d


L2   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 1999 ACS
RN   170277-31-3  REGISTRY
CN   Immunoglobulin G, anti-(human tumor necrosis factor) (human-mouse
     monoclonal cA2 heavy chain), disulfide with human-mouse monoclonal cA2
     light chain, dimer (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Avakine
CN   Infliximab
CN   Remicade
MF   Unspecified
CI   MAN
SR   US Adopted Names Council
LC   STN Files:   ADISINSIGHT, BIOBUSINESS, BIOSIS, CA, CAPLUS, CIN, DRUGNL,
       DRUGPAT, DRUGUPDATES, IPA, PROMT, TOXLINE, TOXLIT

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
              11 REFERENCES IN FILE CA (1967 TO DATE)
              11 REFERENCES IN FILE CAPLUS (1967 TO DATE)


=> file caplus,uspatful,biosis,medline,drugu,embase

COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      10.40     154.71

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                         ENTRY    SESSION
CA SUBSCRIBER PRICE                                       0.00      -2.04

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FILE 'MEDLINE' ENTERED AT 15:32:46 ON 15 SEP 1999

FILE 'DRUGU' ENTERED AT 15:32:46 ON 15 SEP 1999
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FILE 'EMBASE' ENTERED AT 15:32:46 ON 15 SEP 1999
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=> s l1 or l2 or etanercept or infliximab

L3          201 L1 OR L2 OR ETANERCEPT OR INFLIXIMAB
```

```
=> s (tumor necro? factor or tnf)(2a)(antagonist? or inhibit? or block?)

   3 FILES SEARCHED...
L4       17027 (TUMOR NECRO? FACTOR OR TNF)(2A)(ANTAGONIST? OR INHIBIT? OR
               BLOCK?)

=> s l3 and l4

L5          48 L3 AND L4

=> dup rem l5

PROCESSING COMPLETED FOR L5
L6           27 DUP REM L5 (21 DUPLICATES REMOVED)

=> s (neuro? or spin? or cns or nerv?)(2a)(diseas? or disorder? or trauma? or
injur? or damag? or compres? or lesion?) or herniat? disc? or carpal tunnel
or pituitar? adenom? or tumor? or intracran? pressure?

   3 FILES SEARCHED...
   4 FILES SEARCHED...
L7     2267897 (NEURO? OR SPIN? OR CNS OR NERV?)(2A)(DISEAS? OR DISORDER? OR
               TRAUMA? OR INJUR? OR DAMAG? OR COMPRES? OR LESION?) OR
HERNIAT?
               DISC? OR CARPAL TUNNEL OR PITUITAR? ADENOM? OR TUMOR? OR
INTRACR
               AN? PRESSURE?

=> s autoimmun? or ms or multiple sclero? or sclero? panencephalit?

L8      507745 AUTOIMMUN? OR MS OR MULTIPLE SCLERO? OR SCLERO? PANENCEPHALIT?

=> s l3 and (l7 or l8)

L9         165 L3 AND (L7 OR L8)

=> dup rem l9

PROCESSING COMPLETED FOR L9
L10         101 DUP REM L9 (64 DUPLICATES REMOVED)

=> s l10 not py>1998

L11         24 L10 NOT PY>1998

=> s l6 not py>1998

L12          4 L6 NOT PY>1998

=> d 1-4 bib,ab


L12  ANSWER 1 OF 4  CAPLUS  COPYRIGHT 1999 ACS
AN   1998:790259  CAPLUS
DN   130:191248
TI   Etanercept: Antiarthritic TNF-.alpha.
     antagonist
AU   Sorbera, L. A.; Rabasseda, X.; Leeson, P. A.
CS   Prous Science, Barcelona, 08080, Spain
SO   Drugs Future (1998), 23(9), 951-954
     CODEN: DRFUD4; ISSN: 0377-8282
PB   Prous Science
DT   Journal; General Review
LA   English
```

AB A review with 33 refs. of the antiarthritic activity of the fusion protein
(**etanercept**) resulting from the combination of TNF receptor and human IgG1 Fc region.

L12 ANSWER 2 OF 4 CAPLUS COPYRIGHT 1999 ACS
AN 1998:426327 CAPLUS
DN 129:156339
TI Biological agents in rheumatoid arthritis: which ones could be used in combination?
AU Lorenz, Hanns-Martin; Kalden, Joachim R.
CS Department of Medicine III, Institute for Clinical Immunology and Rheumatology, University of Erlangen-Nuremberg, Erlangen, Germany
SO BioDrugs (1998), 9(4), 303-324
CODEN: BIDRF4; ISSN: 1173-8804
PB Adis International Ltd.
DT Journal; General Review
LA English
AB A review with 130 refs. Rheumatoid arthritis is a chronic inflammatory disease. Established treatment is limited because of the clin. response or the induction of adverse effects. New biol. agents evaluated for treatment of rheumatoid arthritis have shown varied clin. success. These agents target cytokines such as tumor necrosis factor-.alpha. (TNF.alpha.), interleukin (IL)-1 or IL-6, or cell surface mols. such as CD4, CD5, CD7, IL-2 receptor, CDw52 or CD54. Amongst these new drugs, only a few have shown clin. effectiveness in double-blind placebo-controlled trials. These include the primatized nondepleting anti-CD4 monoclonal antibody (mAb) CE9.1 (keliximab), the **TNF** .alpha.-**blocking** mAbs cA2 (**infliximab**) and CDP-571, the human recombinant sol. TNF.alpha. receptors p55 (lenercept) and p80, as well as the human recombinant IL-1 receptor antagonist protein, anakinra. Thus, only these agents qualify for evaluation of combination treatment in rheumatoid arthritis. Rationales for combination therapy include: (i) combining drugs with different sites of action to increase efficacy or with different toxicities to minimize risk; (ii) combining drugs with different kinetics, thus improving clin. activity; (iii) using a combination of drugs for the prevention of tachyphylaxis; or (i.v.) using a second drug which helps to prevent or delay the development of resistance to the first one. In addn., combination therapy could help to prevent or minimize adverse effects caused by treatment with biol. agents.
Based on knowledge from trials with biol. agents, and on the different properties attributed to the established disease-modifying antirheumatic drugs (DMARDs) in ex vivo and in vitro studies, we propose evaluation of the following combination regimens involving biol. agents. First, biol. agents targeting TNF.alpha. (such as the mAbs cA2 or CDP-571, or the TNF.alpha. receptor p55-IgG1 fusion protein) given as a single infusion for rapid clin. response could be followed by continuation treatment with methotrexate, possibly combined with chloroquine, azathioprine or cyclosporin. Combination of specific anti-TNF.alpha. strategies with sulfasalazine should be avoided because of the induction of double-stranded DNA antibodies seen after **TNF**.alpha. **blockade** in vivo and reports on a systemic lupus erythematosus-like syndrome as an adverse effect during treatment with biol. agents directed against TNF.alpha. or with sulfasalazine. Alternatively, continuous **inhibition** of **TNF**.alpha. or IL-1 with TNF.alpha. receptor p80-IgG1 fusion protein or IL-1 receptor antagonist, resp., could be combined with methotrexate, with the disadvantage of a slower initial improvement of clin. symptoms. Combination regimens with the primatized CD4 mAb could include methotrexate as concomitant medication, with chloroquine or sulfasalazine as addnl. medication. Importantly, combination of different biol. agents might induce more severe adverse effects than seen with monotherapy. Thus, protocols involving combinations of biol. agents with established DMARDs promise better acceptance than combinations of 2 new and as yet

unestablished drugs with possibly synergistic adverse effects because of their antigenic properties.

L12 ANSWER 3 OF 4 MEDLINE
AN 1999078784 MEDLINE
DN 99078784
TI Update on treatment of rheumatoid arthritis.
AU Schuna A A
CS Rheumatology Clinic, William S. Middleton Veterans Affairs Medical Center,
Madison 53705, USA.. aaschuna@facstaff.wisc.edu
SO JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, (1998 Nov-Dec) 38 (6) 728-35; quiz 735-7. Ref: 20
Journal code: CIL. ISSN: 1086-5802.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
EM 199903
EW 19990301
AB OBJECTIVE: To review current treatment of rheumatoid arthritis (RA), as well as recent advances. DATA SOURCES: MEDLINE search from 1990 to 1998 for human studies using search terms "rheumatoid arthritis"; "cyclooxygenase inhibitors" combined with "anti-inflammatory agents, nonsteroidal"; "**tumor necrosis factor**" limited to "**antagonists** and inhibitors"; "isoxazoles." DATA SYNTHESIS: RA is a chronic inflammatory disease characterized by symmetrical joint involvement, usually of the small joints of the hands and feet. Although the hallmark of the disease is inflammation of joints, other organ systems--including the eyes, blood vessels, lungs, and cardiopulmonary system--may also be involved. Treatment of RA requires both drug and non-drug approaches. Current drug therapy consists of combinations of nonsteroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs). Corticosteroids are also used either for short-term treatment during initiation of therapy, in bursts during acute disease flares, or chronically in low doses. A number of promising new agents are in development. NSAIDs with preferential inhibition of cyclooxygenase II may offer a better safety profile than existing agents. Leflunomide and biological agents such as **etanercept** may provide benefit for patients who fail to achieve adequate response from conventional therapy. CONCLUSION: Traditional approaches to treatment of RA include NSAIDs combined with DMARDs. New agents just reaching the market represent important advances and have the potential to make a positive impact on treatment of RA.

L12 ANSWER 4 OF 4 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 1998394020 EMBASE
TI New drug targets in inflammation and immunomodulation.
AU Norman P.
CS Dr. P. Norman, Norman Consulting, 18 Pink Lane, Burnham, Buckinghamshire SL1 8JW, United Kingdom
SO Drug News and Perspectives, (1998) 11/7 (442-447).
ISSN: 0214-0934 CODEN: DNPEED
CY Spain
DT Journal; Conference Article
FS 030 Pharmacology
037 Drug Literature Index
LA English
SL English
AB A meeting on New Drug Targets in Inflammation and Immunomodulation organized by the Royal Society of Chemistry brought together an international group of scientists. A number of talks on new target elucidation emphasized the huge number of potential drug targets that exist and how signaling pathways converge to use common enzymes.

Pharmacological models such as chemokines, TACE and a novel approach that involves selective inhibition of lymphocyte and leukocyte trafficking will assist in characterizing the roles of newly identified proteins. Several speakers addressed aspects of medicinal chemistry and made presentations on lead generation, structure-related drug design and lead optimization and provided specific examples that cited developmental compounds. The therapeutic utility of new approaches to the treatment of inflammation was illustrated by a description of the antiarthritic effects of the **TNF antagonist etanercept**, which is currently under review as a fast-track product by the U.S. FDA.

=> d l11 1-24 bib,ab

L11 ANSWER 1 OF 24 CAPLUS COPYRIGHT 1999 ACS
AN 1998:790259 CAPLUS
DN 130:191248
TI **Etanercept**: Antiarthritic TNF-.alpha. antagonist
AU Sorbera, L. A.; Rabasseda, X.; Leeson, P. A.
CS Prous Science, Barcelona, 08080, Spain
SO Drugs Future (1998), 23(9), 951-954
CODEN: DRFUD4; ISSN: 0377-8282
PB Prous Science
DT Journal; General Review
LA English
AB A review with 33 refs. of the antiarthritic activity of the fusion protein (**etanercept**) resulting from the combination of TNF receptor and human IgG1 Fc region.

L11 ANSWER 2 OF 24 CAPLUS COPYRIGHT 1999 ACS
AN 1998:766011 CAPLUS
DN 130:181120
TI **Infliximab**: a review of its use in Crohn's disease and rheumatoid arthritis
AU Onrust, Susan V.; Lamb, Harriet M.
CS Adis International Limited, Auckland, N. Z.
SO BioDrugs (1998), 10(5), 397-422
CODEN: BIDRF4; ISSN: 1173-8804
PB Adis International Ltd.
DT Journal; General Review
LA English
AB A review with 96 refs. **Infliximab** is a chimeric monoclonal antibody which binds to and inhibits the activity of **tumor** necrosis factor-.alpha., a cytokine involved in the development of Chrohn's disease and rheumatoid arthritis. In patients with treatment-resistant Crohn's disease, **infliximab** was significantly more effective than placebo in the relief of symptoms. **Infliximab** achieved a clin. response in 44 to 81% of patients with refractory rheumatoid arthritis. Anti-**infliximab** and anti-double-stranded DNA antibodies have developed in some patients, particularly those who received multiple infusions of **infliximab**. In conclusion, **Infliximab** appears to be an effective therapy for patients with treatment-resistant or fistulizing Chrohn's disease or refractory rheumatoid arthritis. The tolerability, long term efficacy and optimal dosage regimen need to be further defined in comparative trials before the full potential of **infliximab** is realized in these patients.

L11 ANSWER 3 OF 24 CAPLUS COPYRIGHT 1999 ACS
AN 1998:741801 CAPLUS

DN 130:152136
TI New biotechnological therapies for Crohn's disease: where are we now?
AU Yacyshyn, Bruce R.
CS University of Alberta, Edmonton, AB, Can.
SO BioDrugs (1998), 10(4), 301-316
CODEN: BIDRF4; ISSN: 1173-8804
PB Adis International Ltd.
DT Journal; General Review
LA English
AB A review with 150 refs. Recent advances in mucosal immunol. have provoked recent interest in the application of biodrugs to Crohn's disease intestinal inflammation. Our understanding of the roles of cytokines, adhesion mols., cell trafficking and cellular immune mechanisms of disease has lead to a no. of recent clin. trials. There has been simultaneous research by a no. of groups using several animal models of gut inflammation. Better animal models and in particular, the use of gene knock-outs and transgenics has benefitted our understanding of the inflammatory components of Crohn's. Examples of early cellular biodrugs included anti-CD4 monoclonal antibodies. The recent FDA approval of **infliximab** was preceded by knowledge that levels of **tumor** necrosis factor (TNF)-.alpha. are suppressed in the clin. therapy of Crohn's. A recent study of interleukin (IL)-10 was not as favorably reported. Equivocal data has not supported the use of IL-10 for Crohn's disease therapy at this time. An ongoing multicenter phase III study of antisense to ICAM-1 for Crohn's disease is nearing completion and review. The newer monoclonal antibodies to enter into clin. studies of Crohn's disease therapy include those targeting the adhesion mols. .beta.7 and .alpha.4.beta.7. Numerous other biodrugs are in planning stages or early clin. studies. The coagulation pathway is another target that has been identified. This range of compds. will produce mixed efficacy in clin. studies and winners and losers will result. Moreover, combination therapy using 2 or more of these and other pre-existing compds. may prove clin. beneficial. Tailoring of pharmaceutical use to specific patients may also be expected, as we understand more about the subclasses of Crohn's disease.

L11 ANSWER 4 OF 24 CAPLUS COPYRIGHT 1999 ACS
AN 1998:736596 CAPLUS
DN 130:94049
TI New **tumor** necrosis factor-.alpha. biologic therapies for rheumatoid arthritis
AU Breedveld, Ferdinand
CS Department of Rheumatology, Leiden University Hospital, Leiden, 2300 RC, Neth.
SO Eur. Cytokine Network (1998), 9(3), 233-238
CODEN: ECYNEJ; ISSN: 1148-5493
PB John Libbey Eurotext
DT Journal; General Review
LA English
AB A review with 39 refs. Continued research towards new and better tolerated therapies to attenuate the inflammation and pain assocd. with rheumatoid arthritis and to halt the progression of erosive joint damage has led to the development of anticytokine strategies. Of these therapies, the most promising appear to be those targeted towards blocking the effects of TNF-.alpha.. Trials with **etanercept** (sTNFR:Fc), which showed significant, rapid, and sustained redns. in disease activity, have produced particularly encouraging results.

L11 ANSWER 5 OF 24 CAPLUS COPYRIGHT 1999 ACS

AN 1998:680268 CAPLUS
DN 130:231664
TI Combination therapy in mice: what can we learn that may be useful for understanding rheumatoid arthritis?
AU Williams, Richard O.
CS Kennedy Institute of Rheumatology, London, W6 8LH, UK
SO Springer Semin. Immunopathol. (1998), 20(1-2), 165-180
CODEN: SSIMDV; ISSN: 0344-4325
PB Springer-Verlag
DT Journal; General Review
LA English
AB A review with 78 refs. There is a growing appreciation of the value of early therapeutic intervention in rheumatoid arthritis (RA) using drugs capable of modifying the disease process, particularly in patients with a poor prognosis. However, most immunomodulatory drugs used to treat RA have toxic side-effects and there is often a relatively small margin between the dose that controls the disease effectively and the dose that produces toxicity. It may be possible to expand the margin between efficacy and toxicity by using combinations of drugs at lower doses than are conventionally used in monotherapy. The arrival of biol. therapies, such as anti-TNF-.alpha. monoclonal antibody (mAb), adds another dimension to the scene by allowing the use of drug/antibody combinations. In fact, clin. trials of drug/antibody combination therapy are already in progress. For example, a recent trial of combination therapy in RA has revealed a synergistic therapeutic effect between the anti-TNF-.alpha. mAb, cA2 (Avakine) and methotrexate. Thus, the duration of the therapeutic effect of low dose cA2 (1 mg/kg) was significantly increased by low-dose methotrexate (7.5 mg/wk) and was accompanied by a marked redn. in the no. of patients with an anti-globulin response to the chimeric TNF-.alpha.-specific antibody. The development of an optimal form of combination therapy for RA will require greater knowledge than is currently available of the mechanisms of action of new, as well as established, therapeutic agents. Thus, synergy between two therapeutic agents is most likely to occur if the agents affect different pathways in the disease process, e.g. the immune and inflammatory pathways. Animal models of arthritis will provide valuable tools, firstly, to det. how different drugs mediate their effects and secondly, to evaluate the efficacy of novel forms of combination therapy and analyze the mechanisms of drug interactions.

L11 ANSWER 6 OF 24 CAPLUS COPYRIGHT 1999 ACS
AN 1998:644276 CAPLUS
DN 130:51133
TI Therapeutic efficacy of multiple intravenous infusions of anti-**tumor** necrosis factor .alpha. monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis
AU Maini, Ravinder N.; Breedveld, Ferdinand C.; Kalden, Joachim R.; Smolen, Josef S.; Davis, Diana; Macfarlane, John D.; Antoni, Christian; Leeb, Burkhard; Elliott, Michael J.; Woody, James N.; Schaible, Thomas F.; Feldmann, Marc
CS FRCP, London, W6 8LH, UK
SO Arthritis Rheum. (1998), 41(9), 1552-1563
CODEN: ARHEAW; ISSN: 0004-3591
PB Lippincott Williams & Wilkins
DT Journal
LA English
AB The authors evaluated the efficacy, pharmacokinetics, immunogenicity, and safety of multiple infusions of a chimeric monoclonal anti-**tumor** necrosis factor .alpha. antibody (cA2) (**infliximab**; Remicade, Centocor, Malvern, PA) given alone or in combination with low-dose methotrexate (MTX) in rheumatoid arthritis (RA) patients. In a 26-wk, double-blind, placebo-controlled, multicenter trial, 101 patients with active RA exhibiting an incomplete response or flare of disease activity

while receiving low-dose MTX were randomized to 1 of 7 groups of 14-15 patients each. The patients received either i.v. cA2 at 1, 3, or 10 mg/kg, with or without MTX 7.5 mg/wk, or i.v. placebo plus MTX 7.5 mg/wk at weeks 0, 2, 6, 10, and 14 and were followed up through week 26. Approx. 60% of patients receiving cA2 at 3 or 10 mg/kg with or without MTX achieved the 20% Paulus criteria for response to treatment, for a median duration of 10.4 to > 18.1 wk ($P < 0.001$ vs. placebo). Patients receiving cA2 at 1 mg/kg without MTX became unresponsive to repeated infusions of cA2 (median duration 2.6 wk; $P = 0.126$ vs. placebo). However, coadministration of cA2 at 1 mg/kg with MTX appeared to be synergistic, prolonging the duration of the 20% response in >60% of patients to a median of 16.5 wk ($P < 0.001$ vs. placebo; $P = 0.006$ vs. no MTX) and the 50% response to 12.2 wk ($P < 0.001$ vs. placebo; $P = 0.002$ vs. no MTX). Patients receiving placebo infusions plus suboptimal low-dose MTX continued to have active disease, with a Paulus response lasting a median of 0 wk. A 70-90% redn. in the swollen joint count, tender joint count, and C-reactive protein level was maintained for the entire 26 wk in patients receiving 10 mg/kg of cA2 with MTX. In general, treatment was well tolerated and stable blood levels of cA2 were achieved in all groups, except for the group receiving 1 mg/kg of cA2 alone, at which dosage antibodies to cA2 were obsd. in .apprx.50% of the patients. Multiple infusions of cA2 were effective and well tolerated, with the best results occurring at 3 and 10 mg/kg either alone or in combination with MTX in .apprx.60% of patients with active RA despite therapy with low-dose MTX. When cA2 at 1 mg/kg was given with low-dose MTX, synergy was obsd. The results of the trial provide a strategy for further evaluation of the efficacy and safety of longer-term treatment with cA2.

L11 ANSWER 7 OF 24 CAPLUS COPYRIGHT 1999 ACS
AN 1998:640165 CAPLUS
DN 130:50954
TI The future role of anti-**tumor** necrosis factor-.alpha. products in the treatment of Crohn's disease
AU Van Hogezand, Ruud A.; Verspaget, Hein W.
CS Department of Gastroenterology-Hepatology, Leiden University Medical Center, Leiden, Neth.
SO Drugs (1998), 56(3), 299-305
CODEN: DRUGAY; ISSN: 0012-6667
PB Adis International Ltd.
DT Journal; General Review
LA English
AB A review with 28 refs. **Tumor** necrosis factor-.alpha. (TNF.alpha.) is thought to play a central role in the immunopathol. of Crohn's disease, particularly since its levels are raised in all types of cells, tissues and secretory fluids of these patients and in animal models of the disease. In addn., TNF.alpha. has been found to modulate a no. of different processes within the network of inflammatory reactions and therefore has become a target mol. for intervention studies. In the past few years several compds. have been developed which neutralize or impair the prodn. of TNF.alpha., e.g. monoclonal antibodies [**infliximab** (cA2), CDP-571], TNF receptor p75-Fc fusion protein, pentoxifylline (oxpentifylline), p65 antisense oligonucleotides and metalloproteinase inhibitors, thereby counteracting the deleterious effects of this proinflammatory cytokine. At present, successful treatment of active 'refractory' and fistulizing Crohn's disease has been reported with anti-TNF.alpha. antibodies; more clin. studies are in progress or will be performed with substances that intervene in the activation, prodn. and processing of TNF.alpha.. Although important aspects of this type of immune-intervention therapy still need to be elucidated, e.g. long term effects, mechanism(s) of action, identification of responders and non-responders, etc., it is obvious that the integration of basic and

clin. research brings a new era of specific cytokine-directed therapy in Crohn's disease.

L11 ANSWER 8 OF 24 CAPLUS COPYRIGHT 1999 ACS
AN 1998:426327 CAPLUS
DN 129:156339
TI Biological agents in rheumatoid arthritis: which ones could be used in combination?
AU Lorenz, Hanns-Martin; Kalden, Joachim R.
CS Department of Medicine III, Institute for Clinical Immunology and Rheumatology, University of Erlangen-Nuremberg, Erlangen, Germany
SO BioDrugs (1998), 9(4), 303-324
CODEN: BIDRF4; ISSN: 1173-8804
PB Adis International Ltd.
DT Journal; General Review
LA English
AB A review with 130 refs. Rheumatoid arthritis is a chronic inflammatory disease. Established treatment is limited because of the clin. response or the induction of adverse effects. New biol. agents evaluated for treatment of rheumatoid arthritis have shown varied clin. success. These agents target cytokines such as **tumor** necrosis factor-.alpha. (TNF.alpha.), interleukin (IL)-1 or IL-6, or cell surface mols. such as CD4, CD5, CD7, IL-2 receptor, CDw52 or CD54. Amongst these new drugs, only a few have shown clin. effectiveness in double-blind placebo-controlled trials. These include the primatized nondepleting anti-CD4 monoclonal antibody (mAb) CE9.1 (keliximab), the TNF.alpha.-blocking mAbs cA2 (**infliximab**) and CDP-571, the human recombinant sol. TNF.alpha. receptors p55 (lenercept) and p80, as well as the human recombinant IL-1 receptor antagonist protein, anakinra. Thus, only these agents qualify for evaluation of combination treatment in rheumatoid arthritis. Rationales for combination therapy include: (i) combining drugs with different sites of action to increase efficacy or with different toxicities to minimize risk; (ii) combining drugs with different kinetics, thus improving clin. activity; (iii) using a combination of drugs for the prevention of tachyphylaxis; or (i.v.) using a second drug which helps to prevent or delay the development of resistance to the first one. In addn., combination therapy could help to prevent or minimize adverse effects caused by treatment with biol. agents.
Based on knowledge from trials with biol. agents, and on the different properties attributed to the established disease-modifying antirheumatic drugs (DMARDs) in ex vivo and in vitro studies, we propose evaluation of the following combination regimens involving biol. agents. First, biol. agents targeting TNF.alpha. (such as the mAbs cA2 or CDP-571, or the TNF.alpha. receptor p55-IgG1 fusion protein) given as a single infusion for rapid clin. response could be followed by continuation treatment with methotrexate, possibly combined with chloroquine, azathioprine or cyclosporin. Combination of specific anti-TNF.alpha. strategies with sulfasalazine should be avoided because of the induction of double-stranded DNA antibodies seen after TNF.alpha. blockade in vivo and reports on a systemic lupus erythematosus-like syndrome as an adverse effect during treatment with biol. agents directed against TNF.alpha. or with sulfasalazine. Alternatively, continuous inhibition of TNF.alpha. or IL-1 with TNF.alpha. receptor p80-IgG1 fusion protein or IL-1 receptor antagonist, resp., could be combined with methotrexate, with the disadvantage of a slower initial improvement of clin. symptoms. Combination regimens with the primatized CD4 mAb could include methotrexate as concomitant medication, with chloroquine or sulfasalazine as addnl. medication. Importantly, combination of different biol. agents might induce more severe adverse effects than seen with monotherapy. Thus, protocols involving combinations of biol. agents with established DMARDs promise better acceptance than combinations of 2 new and as yet unestablished drugs with possibly synergistic adverse effects because of their antigenic properties.

L11 ANSWER 9 OF 24 CAPLUS COPYRIGHT 1999 ACS
AN 1997:752276 CAPLUS
DN 128:46927
TI Recombinant human **tumor** necrosis factor receptor (p75) Fc fusion protein (TNFR:Fc) in rheumatoid arthritis
AU Murray, Kim M.; Dahl, Stephen L.
CS Immunex Corporation, Seattle, WA, 98101, USA
SO Ann. Pharmacother. (1997), 31(11), 1335-1338
CODEN: APHRER; ISSN: 1060-0280
PB Harvey Whitney Books Co.
DT Journal; General Review
LA English
AB A review and discussion, with 25 refs., on the preclin., phase I and phase II data on TNFR:Fc in rheumatoid arthritis. All available data on TNFR:Fc in rheumatoid arthritis are reviewed. Preliminary data indicate that TNFR:Fc is an excellent candidate for future long-term studies in the treatment of rheumatoid arthritis.

L11 ANSWER 10 OF 24 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1999:155065 BIOSIS
DN PREV199900155065
TI Anti-TNFalpha therapy is useful in rheumatoid arthritis and Crohn's disease: Analysis of the mechanism of action predicts utility in other diseases.
AU Feldman, M. (1); Taylor, P.; Paleolog, E.; Brennan, F. M.; Maini, R. N.
CS (1) Kennedy Inst. Rheumatology, 1 Aspenlea Road, Hammersmith, London W6 8LH UK
SO Transplantation Proceedings, (Dec., 1998) Vol. 30, No. 8, pp. 4126-4127.
Meeting Info.: Third International Conference on New Trends in Clinical and Experimental Immunosuppression Geneva, Switzerland February 12-15, 1998
ISSN: 0041-1345.
DT Conference
LA English

L11 ANSWER 11 OF 24 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1999:13810 BIOSIS
DN PREV199900013810
TI Advances in the management of Crohn's disease: Economic and clinical potential of **infliximab**.
AU Hanauer, Stephen B. (1); Cohen, Russell D. (1); Becker, Russell V., III; Larson, Leanne R.; Vreeland, Mary Glenn
CS (1) Sect. Gastroenterol., Dep. Med., Univ. Chicago Med. Cent., Chicago, IL USA
SO Clinical Therapeutics, (Sept.-Oct., 1998) Vol. 20, No. 5, pp. 1009-1028.
ISSN: 0149-2918.
DT General Review
LA English
AB New therapies for Crohn's disease are being developed based on improvements in our understanding of the disease's immune and inflammatory properties. One of these new therapies is **infliximab**, a monoclonal antibody directed against the proinflammatory cytokine **tumor** necrosis factor-alpha. Recent studies indicate that treatment of moderately to severely ill Crohn's disease patients with **infliximab** produces a rapid and profound reduction in the signs, symptoms, and severity of this disease. Beyond its clinical impact, Crohn's disease also carries significant economic consequences. Earlier reports on the costs of managing this disease estimated the average annual medical costs per patient at dollar sign9197, with the total annual cost

of illness estimated to exceed dollar sign1.7 billion. Hospitalizations and surgeries represented 80% of these costs. Additional analyses have been conducted for this review to reflect more current treatment patterns.

Assuming that proven increases in response and remission rates lead to diminished disease severity, **infliximab** can be expected to reduce the number of hospitalizations and surgeries in moderately to severely ill patients, with substantial cost savings. Moreover, improvement in disease status and quality of life may allow Crohn's disease patients to lead more productive lives.

L11 ANSWER 12 OF 24 MEDLINE
AN 1999087732 MEDLINE
DN 99087732
TI **Etanercept** marketed for moderate, severe rheumatoid arthritis [news].
AU Anonymous
SO AMERICAN JOURNAL OF HEALTH-SYSTEM PHARMACY, (1998 Dec 15) 55 (24) 2593. Journal code: CBH. ISSN: 1079-2082.
CY United States
DT (CLINICAL TRIAL)
News Announcement
LA English
FS Priority Journals
EM 199906
EW 19990603

L11 ANSWER 13 OF 24 MEDLINE
AN 1999078784 MEDLINE
DN 99078784
TI Update on treatment of rheumatoid arthritis.
AU Schuna A A
CS Rheumatology Clinic, William S. Middleton Veterans Affairs Medical Center,
Madison 53705, USA.. aaschuna@facstaff.wisc.edu
SO JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, (1998 Nov-Dec) 38 (6) 728-35; quiz 735-7. Ref: 20
Journal code: CIL. ISSN: 1086-5802.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
EM 199903
EW 19990301
AB OBJECTIVE: To review current treatment of rheumatoid arthritis (RA), as well as recent advances. DATA SOURCES: MEDLINE search from 1990 to 1998 for human studies using search terms "rheumatoid arthritis"; "cyclooxygenase inhibitors" combined with "anti-inflammatory agents, nonsteroidal"; "**tumor** necrosis factor" limited to "antagonists and inhibitors"; "isoxazoles." DATA SYNTHESIS: RA is a chronic inflammatory disease characterized by symmetrical joint involvement, usually of the small joints of the hands and feet. Although the hallmark of the disease is inflammation of joints, other organ systems--including the eyes, blood vessels, lungs, and cardiopulmonary system--may also be involved. Treatment of RA requires both drug and non-drug approaches. Current drug therapy consists of combinations of nonsteroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs). Corticosteroids are also used either for short-term treatment during initiation of therapy, in bursts during acute disease flares, or chronically in low doses. A number of promising new agents are in development. NSAIDs with preferential inhibition of cyclooxygenase II may offer a better safety profile than existing agents. Leflunomide and biological agents such as **etanercept** may provide benefit for

patients who fail to achieve adequate response from conventional therapy. CONCLUSION: Traditional approaches to treatment of RA include NSAIDs combined with DMARDs. New agents just reaching the market represent important advances and have the potential to make a positive impact on treatment of RA.

L11 ANSWER 14 OF 24 DRUGU COPYRIGHT 1999 DERWENT INFORMATION LTD
AN 1997-28458 DRUGU T
TI Reduction in serum VEGF and sICAM-1 are associated with clinical response to anti-TNF therapy in patients with severe Crohn's disease.
AU Shealy D; Mace K; DeWoody K; Leone A; DeRita R; Ghrayeb J
CS Centocor
LO Malvern, Pa., USA
SO Gastroenterology (112, No. 4, Suppl., A1090, 1997) 1 Tab.
CODEN: GASTAB ISSN: 0016-5085
AV Centocor Inc., Malvern, Pa., U.S.A.
LA English
DT Journal
FA AB; LA; CT
FS Literature
AB Treatment of severe Crohn's disease (CD) with a chimeric anti-TNFalpha antibody (cA2, **infliximab**) significantly reduces the level of disease activity as assessed by the CD activity index (CDAI). This double-blind, placebo-controlled trial evaluated serum markers associated with inflammation to determine the relationship between their concentration and response to cA2 treatment in 108 patients. These results suggest that clinical response to cA2 treatment is associated with reductions in serum concentrations of VEGF and sICAM-1. Hence, angiogenesis induced by VEGF and cell trafficking mediated by ICAM-1 may be components of CD that are diminished by neutralization of TNFalpha. (conference abstract).

L11 ANSWER 15 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 1999119691 EMBASE
TI Can an anticytokine effectively fight congestive heart failure?.
AU Soran O.; Schneider V.M.; Feldman A.M.
CS Dr. O. Soran, Cardiovasc. Institute Health System, Univ. of Pittsburgh Medical Center, Pittsburgh, PA, United States
SO Cardiology Review, (1998) 15/12 (53-54).
Refs: 4
ISSN: 1092-6607 CODEN: CARRFT
CY United States
DT Journal; (Short Survey)
FS 006 Internal Medicine
018 Cardiovascular Diseases and Cardiovascular Surgery
037 Drug Literature Index
038 Adverse Reactions Titles
LA English

L11 ANSWER 16 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 1999063532 EMBASE
TI Aggressive treatment of early rheumatoid arthritis to prevent joint damage.
AU Pincus T.
CS Dr. T. Pincus, Vanderbilt Univ. School of Medicine, Nashville, TN, United States
SO Bulletin on the Rheumatic Diseases, (1998) 47/8 (2-7).
ISSN: 0007-5248 CODEN: BRDIAZ
CY United States
DT Journal; General Review
FS 030 Pharmacology
031 Arthritis and Rheumatism
037 Drug Literature Index

038 Adverse Reactions Titles
LA English

L11 ANSWER 17 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 1999063531 EMBASE
TI Note from the editor.
AU Conn D.L.
CS Dr. D.L. Conn, Arthritis Foundation, 1330 West Peachtreet, Atlanta, GA 30309, United States
SO Bulletin on the Rheumatic Diseases, (1998) 47/8 (1).
ISSN: 0007-5248 CODEN: BRDIAZ
CY United States
DT Journal; Editorial
FS 030 Pharmacology
031 Arthritis and Rheumatism
037 Drug Literature Index
LA English

L11 ANSWER 18 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 1998400843 EMBASE
TI New drugs for rheumatoid arthritis.
SO Medical Letter on Drugs and Therapeutics, (20 Nov 1998) 40/1040 (110-112).
ISSN: 0025-732X CODEN: MELEAP
CY United States
DT Journal; (Short Survey)
FS 031 Arthritis and Rheumatism
037 Drug Literature Index
038 Adverse Reactions Titles
LA English

L11 ANSWER 19 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 1998371989 EMBASE
TI New genetically engineered drugs promise targeted patient care.
AU Beavers N.
SO Drug Topics, (5 Oct 1998) 142/19 (73-80).
ISSN: 0012-6616 CODEN: DGTNA7
CY United States
DT Journal; General Review
FS 006 Internal Medicine
016 Cancer
037 Drug Literature Index
LA English

L11 ANSWER 20 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 1998371696 EMBASE
TI Biological insights from clinical trials with anti-TNF therapy.
AU Feldmann M.; Charles P.; Taylor P.; Maini R.N.
CS M. Feldmann, Kennedy Institute of Rheumatology, 1 Aspenlea Road, London W6
8LW, United Kingdom
SO Springer Seminars in Immunopathology, (1998) 20/1-2 (211-228).
Refs: 67
ISSN: 0344-4325 CODEN: SSIMDV
CY Germany
DT Journal; Article
FS 026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English

L11 ANSWER 21 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 1998357409 EMBASE
TI Anti-inflammatory inflames Centocor's profits.
AU Glaser V.
SO Nature Biotechnology, (1998) 16/10 (900).

ISSN: 1087-0156 CODEN: NABIF
CY United States
DT Journal; (Short Survey)
FS 026 Immunology, Serology and Transplantation
036 Health Policy, Economics and Management
037 Drug Literature Index
048 Gastroenterology
LA English

L11 ANSWER 22 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 1998332738 EMBASE
TI Clinical value of the detection of antibodies in the serum for diagnosis and treatment of inflammatory bowel disease.
AU Rutgeerts P.
CS Dr. P. Rutgeerts, Division of Gastroenterology, Department of Medicine, University of Leuven, 3000 Leuven, Belgium
SO Gastroenterology, (1998) 115/4 (1006-1022).
Refs: 24
ISSN: 0016-5085 CODEN: GASTAB
CY United States
DT Journal; Editorial
FS 026 Immunology, Serology and Transplantation
037 Drug Literature Index
048 Gastroenterology
LA English

L11 ANSWER 23 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 1998022767 EMBASE
TI [Combination therapy in rheumatic arthritis: New monoclonal antibodies]. NEUER MONOKLONALER ANTIKORPER. DER NEUE MONIKLONALE ANTIKORPER **INFLIXIMAB** (AVAKINE(TM), CA2) ZEIGT BEHANDLUNGSERFOLGE BEI RHEUMATOIDER ARTHRITIS.
SO Deutsche Apotheker Zeitung, (11 Dec 1997) 137/50 (49-50).
ISSN: 0011-9857 CODEN: DAZEA2
CY Germany
DT Journal; Note
FS 026 Immunology, Serology and Transplantation
030 Pharmacology
031 Arthritis and Rheumatism
037 Drug Literature Index
LA German
SL German

L11 ANSWER 24 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
AN 97309212 EMBASE
DN 1997309212
TI A short-term study of chimeric monoclonal antibody cA2 to **tumor** necrosis factor .alpha. for Crohn's Disease.
AU Targan S.R.; Hanauer S.B.; Van Deventer S.J.H.; Mayer L.; Present D.H.; Braakman T.; Dewoody K.L.; Schaible T.F.; Rutgeerts P.J.
CS Dr. S.R. Targan, Cedars-Sinai Medical Center, Division of Gastroenterology, Inflammatory Bowel Dis. Center D4063, 8700 Beverly Blvd., Los Angeles, CA 90048, United States
SO New England Journal of Medicine, (1997) 337/15 (1029-35).
Refs: 36
ISSN: 0028-4793 CODEN: NEJMAG
CY United States
DT Journal; Article
FS 006 Internal Medicine
037 Drug Literature Index
048 Gastroenterology
LA English
SL English
AB Background: Studies in animals and an open-label trial have suggested a role for antibodies to **tumor** necrosis factor .alpha.,

specifically chimeric monoclonal antibody cA2, in the treatment of Crohn's disease. Methods: We conducted a 12-week multicenter, double-blind, placebo-controlled trial of cA2 in 108 patients with moderate-to-severe Crohn's disease that was resistant to treatment. All had scores on the Crohn's Disease Activity Index between 220 and 400 (scores can range from 0 to about 600, with higher scores indicating more severe illness). Patients were randomly assigned to receive a single two-hour intravenous infusion of either placebo or cA2 in a dose of 5 mg per kilogram of body weight, 10 mg per kilogram, or 20 mg per kilogram. Clinical response, the primary end point, was defined as a reduction of 70 or more points in the score on the Crohn's Disease Activity Index at four weeks that was not accompanied by a change in any concomitant medications. Results: At four weeks, 81 percent of the patients given 5 mg of cA2 per kilogram (22 of 27 patients), 50 percent of those given 10 mg of cA2 per kilogram (14 of 28), and 64 percent of those given 20 mg of cA2 per kilogram (18 of 28) had had a clinical response, as compared with 17 percent of patients in the placebo group (4 of 24) ($P<0.001$ for the comparison of the cA2 group as a whole with placebo). Thirty-three percent of the patients given cA2 went into remission (defined as a score below 150 on the Crohn's Disease Activity Index), as compared with 4 percent of the patients given placebo ($P= 0.005$). At 12 weeks, 41 percent of the cA2-treated patients (34 of 83) had had a clinical response, as compared with 12 percent of the patients in the placebo group (3 of 25) ($P = 0.008$). The rates of adverse effects were similar in the groups. Conclusions A single infusion of cA2 was an effective short-term treatment in many patients with moderate-to-severe, treatment-resistant Crohn's disease.

Pat. No. 5605690 printed in FULL format.

5,605,690

<=2> GET 1st DRAWING SHEET OF 7

Feb. 25, 1997

Methods of lowering active TNF- alpha levels in mammals using tumor necrosis factor receptor

TNF
ARTHRITIS

INVENTOR: Jacobs, Cindy A., Seattle, Washington
Smith, Craig A., Seattle, Washington

ASSIGNEE-AT-ISSUE: Immunex Corporation, Seattle, Washington (02)

APPL-NO: 385,229

FILED: Feb. 8, 1995

REL-US-DATA:
Continuation of Ser. No. 946,236, Sep. 15, 1992 now abandoned Which is a continuation-in-part of Ser. No. 523,635, May 10, 1990 now patented 5,395,760 Which is a continuation-in-part of Ser. No. 421,417, Oct. 13, 1989 now abandoned Which is a continuation-in-part of Ser. No. 405,370, Sep. 11, 1989 now abandoned Which is a continuation-in-part of Ser. No. 403,241, Sep. 5, 1989 now abandoned

INT-CL: [6] A61K 39#395; A61K 38#00; C12P 21#04; C07K 14#715

US-CL: 424#134.1; 435#69.7; 514#12; 514#825; 530#350; 530#387.3; 530#866; 530#868;

CL: 424;435;514;530;

SEARCH-FLD: 435#69.1, 69.7, 172.3, 240.27; 424#85.1, 134.1; 530#351, 387.3, 868; 935#9, 12, 15

REF-CITED:

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4,770,995	9/1988	*	Rubin et al.	436#544
5,116,964	5/1992	*	Capon et al.	536#27
5,512,544	4/1996	*	Wallach et al.	
		FOREIGN PATENT DOCUMENTS		
0308378	6/1989	*	European Patent Office (EPO)	C12#N1.500
0422339	7/1990	*	European Patent Office (EPO)	C12#N1.512
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ASST-EXMR: Lucas, John

LEGAL-REP: Malaska; Stephen L.

ABST:
A method for treating TNF-dependent inflammatory diseases in a mammal by administering a TNF antagonist, such as soluble TNFR.

NO-OF-CLAIMS: 6

EXMPL-CLAIM: <=7> 1

NO-OF-FIGURES: 7

NO-DRWNG-PP: 7

PARCASE:
CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation of application Ser. No. 07/946,236, filed Sep. 5, 1992, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 523,635, filed May 10, 1990, now U.S. Pat. No. *5,345,760*, which is a continuation-in-part of U.S. application Ser. No. 421,417, filed Oct. 13, 1989, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 405,370, filed Sep. 11, 1989, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 403,241, filed Sep. 5, 1989, now abandoned.

SUM:

BACKGROUND OF THE INVENTION

The present invention relates generally to cytokine receptors and more specifically to a method of using tumor necrosis factor antagonists to suppress TNF-dependent inflammatory diseases.

Tumor necrosis factor- alpha (TNF alpha , also known as cachectin) and tumor necrosis factor- beta (TNF beta , also known as lymphotoxin) are homologous mammalian endogenous secretory proteins capable of inducing a wide variety of effects on a large number of cell types. The great similarities in the structural and functional characteristics of these two cytokines have resulted in their collective description as "TNF." Complementary cDNA clones encoding TNF alpha (Pennica et al., Nature 312:724, 1984) and TNF beta (Gray et al., Nature 312:721, 1984) have been isolated, permitting further structural and biological characterization of TNF.

TNF proteins initiate their biological effect on cells by binding to specific TNF receptor (TNFR) proteins expressed on the plasma membrane of a TNF-responsive cell. Two distinct forms of TNFR are known to exist: Type I TNFR (TNFRI), having a molecular weight of approximately 75 kilodaltons, and Type II TNFR (TNFRII), having a molecular weight of approximately 55 kilodaltons. TNFRI and TNFRII each bind to both TNF alpha and TNF beta . TNFRI and TNFRII have both been molecularly cloned (Smith et al., Science 248:1019, 1990; Loetscher et al., Cell 61:351, 1990 and Schall et al., Cell 61:361, 1990), permitting recombinant expression and purification of soluble TNFR proteins.

Soluble TNF binding proteins from human urine have also been identified (Peetre et al., Eur. J. Haematol. 41:414, 1988; Seckinger et al., J. Exp. Med. 167:1511, 1988; Seckinger et al., J. Biol. Chem. 264:11966, 1989; UK Patent Application, Publ. No. 2 218 101 A to Seckinger et al.; Engelmann et al., J. Biol. Chem. 264:11974, 1989).

TNF antagonists, such as soluble TNFR and TNF binding proteins, bind to TNF and prevent TNF from binding to cell membrane bound TNF receptors. Such proteins may therefore be useful to suppress biological activities caused by TNF.

The role of TNF in mediated inflammatory diseases and the in vivo biological effects of such soluble TNFR and TNF binding protein proteins in suppressing

such TNF-dependent inflammatory diseases have not been fully elucidated and potential therapeutic uses for TNF antagonists have yet to be identified.

SUMMARY OF THE INVENTION

The present invention provides a method of using TNF antagonists to suppress TNF-dependent inflammatory diseases. Specifically, the present invention provides a method of treating a human having arthritis comprising the step of administering a TNF antagonist, such as soluble human TNFR, to a human.

These and other aspects of the present invention will become evident upon reference to the following detailed description.

DRWDESC:
BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the dimeric structure of the recombinant human TNFR/Fc fusion protein. The primary translation product of the plasmid coding for rhu TNFR/Fc is a single molecule of soluble TNFR linked to single chain of Fc derived from human IgG1. Following translation, but prior to secretion, this fusion molecule dimerizes via 3 cysteine residues in the Fc region to form dimeric rhu TNFR/Fc. Boxes denote structural domains of TNFR.

FIG. 2 shows the construction of plasmid pCAVDHFR rhu TNFR/Fc. Abbreviations are as follows: ADH2, yeast alcohol dehydrogenase gene and regulatory region; CMV, cytomegalovirus immediate early enhancer; TPL, adenovirus-2 tripartite leader; VA, adenovirus-2 virus-associated RNA genes I and II; DHFR, hamster dihydrofolate reductase gene.

FIGS. 3 and 4 are graphs showing the effect of intra-articular administration of recombinant human TNFR/Fc, monomeric TNFR, recombinant murine IL-1R and TNFR monomer combined with rmulL-1R on antigen-induced arthritis in rats. The data indicate that TNFR/Fc, TNFR monomer, rmu IL-1R and TNFR combined with IL-1R suppress inflammation associated with antigen-induced arthritis.

FIG. 5 shows the effect of intraperitoneal administration of recombinant human TNFR/Fc and PBS (vehicle control) on the development of collagen induced arthritis (CIA) in B 10.RIII mice. TNFR/Fc significantly delayed the onset of CIA.

FIG. 6 shows the effect of intraperitoneal administration of recombinant human TNFR/Fc and PBS (vehicle control) on the development of collagen induced arthritis (CIA) in DBA/1 mice. TNFR/Fc significantly delayed the onset of CIA.

FIG. 7 shows that administration of TNFR/Fc in mice reduced the arthritis index and the number of joints showing signs of arthritis.

DETDESC:

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the terms "TNF receptor" and "TNFR" refer to proteins having amino acid sequences which are substantially similar to the native mammalian TNF receptor or TNF binding protein amino acid sequences, and which are capable of binding TNF molecules and inhibiting TNF from binding to cell membrane bound TNFR. Two distinct types of TNFR are known to exist: Type I TNFR (TNFRI) and Type II TNFR (TNFRII). The mature full-length human TNFRI is a glycoprotein having a molecular weight of about 75-80 kilodaltons (kDa). The mature full-length human TNFRII is a glycoprotein having a molecular weight of about 55-60 kilodaltons (kDa). The preferred TNFRs of the present invention are soluble forms of TNFRI and TNFRII, as well as soluble TNF binding proteins. Soluble TNFR molecules include, for example, analogs or subunits of native proteins having at least 20 amino acids and which exhibit at least some biological activity in common with TNFRI, TNFRII or TNF binding proteins. Soluble TNFR constructs are devoid of a transmembrane region (and are secreted from the cell) but retain the ability to bind TNF. Various bioequivalent protein and amino acid analogs have an amino acid sequence corresponding to all or part of the extracellular region of a native TNFR, for example, huTNFRI DELTA 235, huTNFRI DELTA 185 and huTNFRI DELTA 163, or amino acid sequences substantially similar to the sequences of amino acids 1-163, amino acids 1-185, or amino acids 1-235 of SEQ ID NO:1, and which are biologically active in that they bind to TNF ligand. Equivalent soluble TNFRs include polypeptides which vary from these sequences by one or more substitutions, deletions, or additions, and which retain the ability to bind TNF or inhibit TNF signal transduction activity via cell surface bound TNF receptor proteins, for example huTNFRI DELTA x, wherein x is selected from the group consisting of any one of amino acids 163-235 of SEQ ID NO:1. Analogous deletions may be made to muTNFR. Inhibition of TNF signal transduction activity can be determined by transfecting cells with recombinant TNFR DNAs to obtain recombinant receptor expression. The cells are then contacted with TNF and the resulting metabolic effects examined. If an effect results which is attributable to the action of the ligand, then the recombinant receptor has signal transduction activity. Exemplary procedures for determining whether a polypeptide has signal transduction activity are disclosed by Idzerda et al., J. Exp. Med. 171:861 (1990); Curtis et al., Proc. Natl. Acad. Sci. U.S.A. 86:3045 (1989); Prywes et al., EMBO J. 5:2179 (1986) and Chou et al., J. Biol. Chem. 262:1842 (1987). Alternatively, primary cells or cell lines which express an endogenous TNF receptor and have a detectable biological response to TNF could also be utilized.

The nomenclature for TNFR analogs as used herein follows the convention of naming the protein (e.g., TNFR) preceded by either hu (for human) or mu (for murine) and followed by a DELTA (to designate a deletion) and the number of the C-terminal amino acid. For example, huTNFR DELTA 235 refers to human TNFR having Asp<235 > as the C-terminal amino acid (i.e., a polypeptide having the sequence of amino acids 1-235 of SEQ ID NO:1). In the absence of any human or murine species designation, TNFR refers generically to mammalian TNFR. Similarly, in the absence of any specific designation for deletion mutants, the term TNFR means all forms of TNFR, including routants and analogs which possess TNFR biological activity.

The term "isolated" or "purified", as used in the context of this specification to define the purity of TNFR protein or protein compositions, means that the protein or protein composition is substantially free of other proteins of natural or endogenous origin and contains less than about 1% by mass of protein contaminants residual of production processes. Such compositions,

however, can contain other proteins added as stabilizers, carders, excipients or co-therapeutics. TNFR is isolated if it is detectable as a single protein band in a polyacrylamide gel by silver staining.

"Recombinant," as used herein, means that a protein is derived from recombinant (e.g., microbial or mammalian) expression systems. "Microbial" refers to recombinant proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product, "recombinant microbial" defines a protein produced in a microbial expression system which is essentially free of native endogenous substances. Protein expressed in most bacterial cultures, e.g., E. coli, will be free of glycan. Protein expressed in yeast may have a glycosylation pattern different from that expressed in mammalian cells.

"Biologically active," as used throughout the specification as a characteristic of TNF receptors, means that a particular molecule shares sufficient amino acid sequence similarity with the embodiments of the present invention disclosed herein to be capable of binding detectable quantities of TNF, transmitting a TNF stimulus to a cell, for example, as a component of a hybrid receptor construct, or cross-reacting with anti-TNFR antibodies raised against TNFR from natural (i.e., nonrecombinant) sources. Preferably, biologically active TNF receptors within the scope of the present invention are capable of binding greater than 0.1 nmoles TNF per nmole receptor, and most preferably, greater than 0.5 nmole TNF per nmole receptor in standard binding assays (see below).

Soluble TNF Antagonists and Analogs

The present invention utilizes isolated and purified TNF antagonist polypeptides. The isolated and purified TNF antagonist polypeptides used in this invention are substantially free of other contaminating materials of natural or endogenous origin and contain less than about 1% by mass of protein contaminants residual of production processes. The TNF antagonist polypeptides used in this invention are optionally without associated native-pattern glycosylation.

In preferred aspects of the present invention, the TNF antagonists are selected from the group consisting of soluble human TNFRI and TNFR II. The pCAV/NOT-TNFR vector, containing the human TNFRI cDNA clone 1, was used to express and purify soluble human TNFRI. pCAV/NOT-TNFR has been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852, U.S.A. (Accession No. 68088) under the name pCAV/NOT-TNFR.

Like most mammalian genes, mammalian TNF receptors are presumably encoded by multi-exon genes. Alternative mRNA constructs which can be attributed to different mRNA splicing events following transcription, and which share large regions of identity or similarity with the cDNAs claimed herein may also be used.

Other mammalian TNFR cDNAs may be isolated by using an appropriate human TNFR DNA sequence as a probe for screening a particular mammalian cDNA library by cross-species hybridization. Mammalian TNFR used in the present invention includes, by way of example, primate, human, murine, canine, feline, bovine, ovine, equine and porcine TNFR. Mammalian TNFRs can be obtained by cross species hybridization, using a single stranded cDNA derived from the human TNFR DNA sequence as a hybridization probe to isolate TNFR cDNAs from mammalian cDNA

libraries.

Derivatives of TNFR which may be used in the present invention also include various structural forms of the primary protein which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, a TNFR protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction.

The primary amino acid structure may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants. Covalent derivatives are prepared by linking particular functional groups to TNFR amino acid side chains or at the N- or C-termini. Other derivatives of TNFR include covalent or aggregative conjugates of TNFR or its fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast alpha -factor leader). TNFR protein fusions can comprise peptides added to facilitate purification or identification of TNFR (e.g., poly-His). The amino acid sequence of TNF receptor can also be linked to the peptide Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (DYKDDDDK) (Hopp et al., Bio/Technology 6:1204,1988.) The latter sequence is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. This sequence is also specifically cleaved by bovine mucosal enterokinase at the residue immediately following the Asp-Lys pairing. Fusion proteins capped with this peptide may also be resistant to intracellular degradation in E. coli.

TNFR with or without associated native-pattern glycosylation may also be used. TNFR expressed in yeast or mammalian expression systems, e.g., COS-7 cells, may be similar or slightly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system. Expression of TNFR DNAs in bacteria such as E. coli provides non-glycosylated molecules. Functional mutant analogs of mammalian TNFR having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A1-Z, where A1 is any amino acid except Pro, and Z is Ser or Thr. In this sequence, Asn provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A1 and Z, or an amino acid other than Asn between Asn and A1.

TNFR derivatives may also be obtained by mutations of TNFR or its subunits. A TNFR mutant, as referred to herein, is a polypeptide homologous to TNFR but which has an amino acid sequence different from native TNFR because of a deletion, insertion or substitution.

Bioequivalent analogs of TNFR proteins may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues can be deleted (e.g., Cys<178>) or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involve modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present. Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those having physiochemical characteristics resembling those of the residue to be replaced. Similarly, when a deletion or insertion strategy is adopted, the potential effect of the deletion or insertion on biological activity should be considered. Substantially similar polypeptide sequences, as defined above, generally comprise a like number of amino acids sequences, although C-terminal truncations for the purpose of constructing soluble TNFRs will contain fewer amino acid sequences. In order to preserve the biological activity of TNFRs, deletions and substitutions will preferably result in homologous or conservatively substituted sequences, meaning that a given residue is replaced by a biologically similar residue. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known. Moreover, particular amino acid differences between human, murine and other mammalian TNFRs is suggestive of additional conservative substitutions that may be made without altering the essential biological characteristics of TNFR.

Subunits of TNFR may be constructed by deleting terminal or internal residues or sequences. Particularly preferred sequences include those in which the transmembrane region and intracellular domain of TNFR are deleted or substituted with hydrophilic residues to facilitate secretion of the receptor into the cell culture medium. The resulting protein is referred to as a soluble TNFR molecule which retains its ability to bind TNF. A particularly preferred soluble TNFR construct is TNFRI DELTA 235 (the sequence of amino acids 1-235 of SEQ ID NO:1), which comprises the entire extracellular region of TNFRI, terminating with Asp<235 > immediately adjacent the transmembrane region. Additional amino acids may be deleted from the transmembrane region while retaining TNF binding activity. For example, huTNFRI DELTA 183 which comprises the sequence of amino acids 1-183 of SEQ ID NO: 1, and TNFRI DELTA 163 which comprises the sequence of amino acids 1-163 of SEQ ID NO: 1, retain the ability to bind TNF ligand. TNFRI DELTA 142, however, does not retain the ability to bind TNF ligand. This suggests that one or both of Cys<157 > and Cys<163 > is required for formation of an intramolecular disulfide bridge for the proper folding of TNFRI. Cys<178> , which was deleted without any apparent adverse effect on the ability of the soluble TNFRI to bind TNF, does not appear to be essential for proper folding of TNFRI. Thus, any deletion C-terminal to Cys<163 > would be expected to result in a biologically active soluble TNFRI. The present invention contemplates use of such soluble TNFR constructs corresponding to all or part of the extracellular region of TNFR terminating with any amino acid after Cys<163> . Other C-terminal deletions, such as TNFRI DELTA 157, may be made as a matter of convenience by cutting TNFR cDNA with appropriate restriction enzymes and, if necessary, reconstructing specific sequences with synthetic oligonucleotide linkers. Soluble TNFR with N-terminal deletions may also be used in the present

invention. For example, the N-terminus of TNFRI may begin with Leu<1> , Pro<2 > or Ala<3 > without significantly affecting the ability of TNFRI to effectively act as a TNF antagonist. The resulting soluble TNFR constructs are then inserted and expressed in appropriate expression vectors and assayed for the ability to bind TNF.

Mutations in nucleotide sequences constructed for expression of analog TNFR must, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures such as loops or hairpins which would adversely affect translation of the receptor mRNA. Although a mutation site may be predetermined, it is not necessary that the nature of the mutation per se be predetermined. For example, in order to select for optimum characteristics of mutants at a given site, random mutagenesis may be conducted at the target codon and the expressed TNFR mutants screened for the desired activity.

Not all mutations in the nucleotide sequence which encodes TNFR will be expressed in the final product, for example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference), or to provide codons that are more readily translated by the selected host, e.g., the well-known E. coli preference codons for E. coli expression.

Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (Gene 42:133, 1986); Bauer et al. (Gene 37:73, 1985); Craik (BioTechniques, Jan. 1985, 12-19); Smith et al. (Genetic Engineering: Principles and Methods, Plenum Press, 1981); and U.S. Pat. Nos. *4,518,584* and *4,737,462* disclose suitable techniques, and are incorporated by reference herein.

Both monovalent forms and polyvalent forms of TNFR may also be used in the present invention. Polyvalent forms possess multiple TNFR binding sites for TNF ligand. For example, a bivalent soluble TNFR may consist of two tandem repeats of amino acids 1-235 of SEQ ID NO:1, separated by a linker region. Alternate polyvalent forms may also be constructed, for example, by chemically coupling TNFR to any clinically acceptable carder molecule, a polymer selected from the group consisting of Ficoll, polyethylene glycol or dextran using conventional coupling techniques. Alternatively, TNFR may be chemically coupled to biotin, and the biotin-TNFR conjugate then allowed to bind to avidin, resulting in tetravalent avidin/biotin/TNFR molecules. TNFR may also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugate precipitated with anti-DNP or anti-TNP-IgM, to form decameric conjugates with a valency of 10 for TNFR binding sites.

A recombinant chimeric antibody molecule may also be produced having TNFR sequences substituted for the variable domains of either or both of the

immunoglobulin molecule heavy and light chains and having unmodified constant region domains. For example, chimeric TNFR/IgG1 may be produced from two chimeric genes-a TNFR/human kappa light chain chimera (TNFR/G kappa) and a TNFR/human gamma 1 heavy chain chimera (TNFR/C gamma - 1). Following transcription and translation of the two chimeric genes, the gene products assemble into a single chimeric antibody molecule having TNFR displayed bivalently. Such polyvalent forms of TNFR may have enhanced binding affinity for TNF ligand. One specific example of a TNFR/Fc fusion protein is disclosed in SEQ ID NO:3 and SEQ ID NO:4. Additional details relating to the construction of such chimeric antibody molecules are disclosed in WO 89/09622 and EP 315062.

Expression of Recombinant TNFR

Recombinant expression vectors are preferably used to amplify or express DNA encoding TNFR to obtain purified TNFR. Recombinant expression vectors are replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding mammalian TNFR or bioequivalent analogs operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences, as described in detail below. Such regulatory elements may include an operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated. DNA regions are operably linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous and in reading frame. Structural elements intended for use in yeast expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

DNA sequences encoding mammalian TNF receptors which are to be expressed in a microorganism will preferably contain no introns that could prematurely terminate transcription of DNA into mRNA; however, premature termination of transcription may be desirable, for example, where it would result in mutants having advantageous C-terminal truncations, for example, deletion of a transmembrane region to yield a soluble receptor not bound to the cell membrane. Due to code degeneracy, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence. Other embodiments include sequences capable of hybridizing to the sequences of the provided cDNA under moderately stringent conditions (50o C., 2 x SSC) and other sequences hybridizing or degenerate to those which encode biologically active TNF receptor

polypeptides.

Recombinant TNFR DNA is expressed or amplified in a recombinant expression system comprising a substantially homogeneous monoculture of suitable host microorganisms, for example, bacteria such as E. coli or yeast such as S. cerevisiae, which have stably integrated (by transformation or transfection) a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit as a component of a resident plasmid. Generally, cells constituting the system are the progeny of a single ancestral transformant. Recombinant expression systems as defined herein will express heterologous protein upon induction of the regulatory elements linked to the DNA sequence or synthetic gene to be expressed.

Transformed host cells are cells which have been transformed or transfected with TNFR vectors constructed using recombinant DNA techniques. Transformed host cells ordinarily express TNFR, but host cells transformed for purposes of cloning or amplifying TNFR DNA do not need to express TNFR. Expressed TNFR will be deposited in the cell membrane or secreted into the culture supernatant, depending on the TNFR DNA selected. Suitable host cells for expression of mammalian TNFR include prokaryotes, yeast or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example E. coli or bacilli. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed to produce mammalian TNFR using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., 1985), the relevant disclosure of which is hereby incorporated by reference.

Prokaryotic expression hosts may be used for expression of TNFR that do not require extensive proteolytic and disulfide processing. Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium, and various species within the genera Pseudomonas, Streptomyces, and Staphyolococcus, although others may also be employed as a matter of choice.

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, Wis. U.S.A.). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. E. coli is typically transformed using derivatives of pBR322, a plasmid derived from an E. coli species (Bolivar et al., Gene 2:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells.

Promoters commonly used in recombinant microbial expression vectors include the beta -lactamase (penicillinase) and lactose promoter system (Chang et al., Nature 275:615, 1978; and Goeddel et al., Nature 281:544, 1979), the tryptophan

(trp) promoter system (Goeddel et al., Nucl. Acids Res. 8:4057, 1980; and EPA 36,776) and tac promoter (Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful bacterial expression system employs the phage lambda P L promoter and cI857ts thermolabile repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the lambda P L promoter include plasmid pHUB2, resident in E. coli. strain JMB9 (ATCC 37092) and pPLc28, resident in E. coli. RR1 (ATCC 53082).

Recombinant TNFR proteins may also be expressed in yeast hosts, preferably from the Saccharomyces species, such as S. cerevisiae. Yeast of other genera, such as Pichia or Kluyveromyces may also be employed. Yeast vectors will generally contain an origin of replication from the 2 mu yeast plasmid or an autonomously replicating sequence (ARS), promoter, DNA encoding TNFR, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable marker permitting transformation of both yeast and E. coli., e.g., the ampicillin resistance gene of E. coli. and S. cerevisiae TRP1 or URA3 gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the TRP1 or URA3 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan or uracil.

Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., J. Biol. Chem. 255:2073, 1980) or other glycolytic enzymes (Hess et al., J. Adv. Enzyme Reg. 7:149, 1968; and Holland et al., Biochem. 7:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657.

Preferred yeast vectors can be assembled using DNA sequences from pUC18 for selection and replication in E. coli. (Amp<r > gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and a-factor secretion leader. The ADH2 promoter has been described by Russell et al. (J. Biol. Chem. 258:2674, 1982) and Beier et al. (Nature 300:724, 1982). The yeast alpha -factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed. See, e.g., Kurjan et al., Cell 30:933, 1982; and Bitter et al., Proc. Natl. Acad. Sci. U.S.A. 81:5330, 1984. The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

Suitable yeast transformation protocols are known to those of skill in the art; an exemplary technique is described by Hinnen et al., Proc. Natl. Acad. Sci. U.S.A. 75:1929, 1978, selecting for Trp< + > transformants in a selective medium consisting of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 mu g/ml adenine and 20 mu g/ml uracil or URA + tranformants in medium consisting of 0.67% YNB, with amino acids and bases as described by Sherman et al., Laboratory Course Manual for Methods in Yeast Genetics, Cold Spring Harbor

Laboratory, Cold Spring Harbor, N.Y., 1986.

Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% or 4% glucose supplemented with 80 mu g/ml adenine and 80 mu g/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4o C. prior to further purification.

Various mammalian or insect cell culture systems are also advantageously employed to express recombinant protein. Expression of recombinant proteins in mammalian cells is particularly preferred because such proteins are generally correctly folded, appropriately modified and completely functional. Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (Cell 23:175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, Bio/Technology 6:47 (1988).

The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the Hind 3 site toward the Bgl1 site located in the vital origin of replication is included. Further, mammalian genomic TNFR promoter, control and/or signal sequences may be utilized, provided such control sequences are compatible with the host cell chosen. Additional details regarding the use of a mammalian high expression vector to produce a recombinant mammalian TNF receptor are provided in Examples 2 and 7 below. Exemplary vectors can be constructed as disclosed by Okayama and Berg (Mol. Cell. Biol. 3:280, 1983).

A useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (Mol. Immunol. 23:935, 1986).

Recombinant expression vectors comprising TNFR cDNAs are stably integrated into a host cell's DNA. Elevated levels of expression product is achieved by selecting for cell lines having amplified numbers of vector DNA. Cell lines having amplified numbers of vector DNA are selected, for example, by transforming a host cell with a vector comprising a DNA sequence which encodes an enzyme which is inhibited by a known drug. The vector may also comprise a DNA

sequence which encodes a desired protein. Alternatively, the host cell may be co-transformed with a second vector which comprises the DNA sequence which encodes the desired protein. The transformed or co-transformed host cells are then cultured in increasing concentrations of the known drug, thereby selecting for drug-resistant cells. Such drug-resistant cells survive in increased concentrations of the toxic drug by over-production of the enzyme which is inhibited by the drug, frequently as a result of amplification of the gene encoding the enzyme. Where drug resistance is caused by an increase in the copy number of the vector DNA encoding the inhibitable enzyme, there is a concomitant co-amplification of the vector DNA encoding the desired protein (TNFR) in the host cell's DNA.

A preferred system for such co-amplification uses the gene for dihydrofolate reductase (DHFR), which can be inhibited by the drug methotrexate (MTX). To achieve co-amplification, a host cell which lacks an active gene encoding DHFR is either transformed with a vector which comprises DNA sequence encoding DHFR and a desired protein, or is co-transformed with a vector comprising a DNA sequence encoding DHFR and a vector comprising a DNA sequence encoding the desired protein. The transformed or co-transformed host cells are cultured in media containing increasing levels of MTX, and those cells lines which survive are selected.

A particularly preferred co-amplification system uses the gene for glutamine synthetase (GS), which is responsible for the synthesis of glutamate and ammonia using the hydrolysis of ATP to ADP and phosphate to drive the reaction. GS is subject to inhibition by a variety of inhibitors, for example methionine sulphoximine (MSX). Thus, TNFR can be expressed in high concentrations by co-amplifying cells transformed with a vector comprising the DNA sequence for GS and a desired protein, or co-transformed with a vector comprising a DNA sequence encoding GS and a vector comprising a DNA sequence encoding the desired protein, culturing the host cells in media containing increasing levels of MSX and selecting for surviving cells. The GS co-amplification system, appropriate recombinant expression vectors and cells lines, are described in the following PCT applications: WO 87/04462, WO 89/01036, WO 89/10404 and WO 86/05807.

Recombinant proteins are preferably expressed by co-amplification of DHFR or GS in a mammalian host cell, such as Chinese Hamster Ovary (CHO) cells, or alternatively in a murine myeloma cell line, such as SP2/0-Ag14 or NS0 or a rat myeloma cell line, such as YB2/3.0-Ag20, disclosed in PCT applications WO/89/10404 and WO 86/05807.

A preferred eukaryotic vector for expression of TNFR DNA is disclosed below in Example 1. This vector, referred to as pCAV/NOT, was derived from the mammalian high expression vector pDC201 and contains regulatory sequences from SV40, adenovirus-2, and human cytomegalovirus.

Purification of Recombinant TNFR

Purified mammalian TNF receptors or analogs are prepared by culturing suitable host/vector systems to express the recombinant translation products of the DNAs of the present invention, which are then purified from culture media or cell extracts.

For example, supernatants from systems which secrete recombinant protein into

culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise a TNF or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a TNFR composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant mammalian TNFR can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express mammalian TNFR as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (J. Chromatog. 296:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human GM-CSF on a preparative HPLC column.

Human TNFR synthesized in recombinant culture is characterized by the presence of non-human cell components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover human TNFR from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of TNFR free of proteins which may be normally associated with TNFR as it is found in nature in its species of origin, e.g. in cells, cell exudates or body fluids.

Therapeutic Administration of Recombinant Soluble TNFR

The present invention provides methods of suppressing TNF-dependent inflammatory responses in humans comprising administering an effective amount of a TNF antagonist, such as TNFR, and a suitable diluent and carrier.

For therapeutic use, purified soluble TNFR protein is administered to a patient, preferably a human, for treatment of arthritis. Thus, for example, soluble TNFR protein compositions can be administered, for example, via intra-articular, intraperitoneal or subcutaneous routes by bolus injection,

continuous infusion, sustained release from implants, or other suitable techniques. Typically, a soluble TNFR therapeutic agent will be administered in the form of a composition comprising purified protein in conjunction with physiologically acceptable carriers, excipients or diluents. Such carriers will be nontoxic to recipients at the dosages and concentrations employed. Ordinarily, the preparation of such compositions entails combining the TNFR with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Appropriate dosages can be determined in trials. In accordance with appropriate industry standards, preservatives may also be added, such as benzyl alcohol. The amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the condition of the patient, and so forth.

TNF antagonist proteins are administered to a mammal, preferably a human, for the purpose treating TNF-dependent inflammatory diseases, such as arthritis. For example, TNFRI proteins inhibit TNF-dependent arthritic responses. Because of the primary roles IL-1 and IL-2 play in the production of TNF, combination therapy using TNFR in combination with IL-1R and/or IL-2R may be preferred in the treatment of TNF-associated clinical indications. In the treatment of humans, soluble human TNFR is preferred. Either Type I IL-1R or Type II IL-1R, or a combination thereof, may be used in accordance with the present invention to treat TNF-dependent inflammatory diseases, such as arthritis. Other types of TNF binding proteins may be similarly used.

For treatment of arthritis, TNFR is administered in systemic amounts ranging from about 0.1 mg/kg/week to about 100 mg/kg/week. In preferred embodiments of the present invention, TNFR is administered in amounts ranging from about 0.5 mg/kg/week to about 50 mg/kg/week. For local intra-articular administration, dosages preferably range from about 0.01 mg/kg to about 1.0 mg/kg per injection.

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

Example 1

Expression and Purification Of Soluble Human TNFRI

The cloning of the cDNA for the 80 kD form of the human TNF receptor has been described in detail (Smith et al., Science 248:1019, 1990). The expression vector pCAV/NOT-TNFR (ATCC 68088) containing the TNFR cDNA clone 1 was used to prepare and express a soluble human TNFRI as follows.

A cDNA encoding a soluble human TNFRI DELTA 235 (the primary translation product of which had the sequence of amino acids -22-235 of SEQ ID NO:1) was

constructed by excising an 840 bp fragment from pCAV/NOT-TNFR with the restriction enzymes Not1 and Pvu2. Not1 cuts at the multiple cloning site of pCAV/NOT-TNFR and Pvu2 cuts within the TNFR coding region 20 nucleotides 5' of the transmembrane region. In order to reconstruct the 3' end of the TNFR sequences, two oligonucleotides were synthesized and annealed to create the following oligonucleotide linker encoding amino acids corresponding to amino acids 229-235 of SEQ ID NO:1: [See Original Patent for Chemical Structure Diagram] GACTTCCCTCGTGACCGCTGATTCCTAGGTCTAGAlaGluGlySerThrGlyAspEnd

This oligonucleotide linker has terminal Pvu2 and Bgl2 restriction sites, regenerates 20 nucleotides of the TNFR, followed by a termination codon (underlined) and a BamH1 restriction site (for convenience in isolating the entire soluble TNFR by Not1/BamH1 digestion). This oligonucleotide was then ligated with the 840 bp Not1/Pvu2 TNFR insert into Bgl2/Not1 cut pCAV/NOT to yield psolhuTNFR DELTA 235/CAVNOT, which was transfected into COS-7 cells as described above. The host cells expressed a mature a soluble human TNFRI protein having the sequence of amino acids 1-235 which was capable of binding TNF.

Example 2

Construction and Expression of Soluble Human TNFR/Fc Fusion Protein

A schematic diagram showing the construction of a recombinant soluble human TNFR:Fc expression vector is shown in FIG. 1. The rhu TNFR:Fc fusion gene was created by ligating the following fragments into Bluescript Registered TM , a commercially available cloning vector (Stratagene):

1) An 867 bp Asp718-Pvu2 fragment from pCAV/NOT-TNFR (ATCC 68088) containing the cDNA encoding the truncated TNFR.

2) A 700 bp Sty1-Spe1 fragment from plasmid pIXY498 coding for 232 amino acids of the Fc portion of human IgG1. Plasmid pIXY498 is a yeast expression vector containing the Fc fragment of human IgG1 (see FIG. 2).

3) An oligonucleotide linker, to fuse the truncated TNFR with the human IgG1 Fc fragment. This linker was created by PCR (polymerase chain reaction) amplification using two primers, one having the sequence CCCCAGCTGAAGGGAGCACTGGCG ACGAGCCCAAATCTTGTGACAAAACTC (nucleotides 833-883 of SEQ ID NO:3) which encodes the 3' end of the truncated TNF receptor and the 5' end of human IgG1, and the other having the sequence CGGTACGTGCTGTTGTTACTGC (SEQ ID NO:5), an antisense sequence encoding nucleotides 257-237 of human IgG1. The template for this reaction was pIXY498. The reaction product was digested with Pvu2 and Sty1, and a 115 bp fragment was isolated.

This construct was then digested with Not1 and the resulting 1.4 kilobase fragment containing the rhu TNFR:Fc fusion DNA sequence was ligated into the Not1 site of plasmid CAV/NOT/DHFR. Plasmid pCAV/NOT/DHFR was derived from plasmid pCAV/NOT by inserting the hamster dihydrofolate reductase DNA sequence (DHFR) into the Hpal site of pCAV/NOT (FIG. 2). This construct was designated plasmid pCAVDHFRhuTNFRFc. The entire coding region sequence was confirmed by DNA

sequencing and is depicted in FIG. 2.

To prepare the host strain, DXB-11 CHO cells deficient in the expression of dihydrofolate reductase (DHFR) were obtained from Dr. Lawren Chasin at Columbia University. A bank of 100 vials of these cells was established, and representative vials were sent to Microbiological Associates for examination via the following procedures:

Test	Result
1. Transmission Electron Microscopy (TEM)	Type A only,
2. Sterility - Bacterial and Fungal	negative
3. Mycoplasma	negative
4. Mouse Antibody Production (MAP)	negative

All transfections and amplification steps were performed in a separate laboratory set aside for this purpose. Only mycoplasma-free cell lines were allowed into this facility.

Transfections were performed by mixing pCAVDHFRhuTNFRFc plasmid DNA with Lipofectin TM reagent from Gibco BRL. Approximately 10 > g of DNA was added to 10 cm petri dishes containing CHO DXB-11 cells. After the initial transfection, cells were selected for the expression of DHFR by subculturing in selective medium lacking glycine, hypoxanthine and thymidine. The resulting colonies were then transferred to 24 well plates and analyzed for rhu TNFR:Fc expression. The highest expressing cultures were subjected to amplification by exposure to increasing concentrations of methotrexate (MTX). Cells able to grow at 25 nM MTX were cloned by limiting dilution in 96 well plates. The highest expressing clones were transferred to suspension culture and the final selection of clone 4-4FC102A5-3 was made based on its high level of rhu TNFR:Fc expression under these conditions.

Example 3

Expression of Monomeric Soluble TNF Receptors in CHO Cells

Soluble TNF receptor was expressed in Chinese Hamster Ovary (CHO) cells using the glutamine-synthetase (GS) gene amplification system, substantially as described in PCT patent application Nos. WO87/04462 and WO89/01036. Briefly, CHO cells are transfected with an expression vector containing genes for both TNFR and GS. CHO cells are selected for GS gene expression based on the ability of the transfected DNA to confer resistance to low levels of methionine sulphoximine (MSX). GS sequence amplification events in such cells are selected using elevated MSX concentrations. In this way, contiguous TNFR sequences are also amplified and enhanced TNFR expression is achieved.

The vector used in the GS expression system was psolTNFR/P6/PSVLGS, which was constructed as follows. First, the vector pSVLGS.1 (described in PCT Application Nos. WO87/04462 and WO89/01036, and available from Celltech, Ltd., Berkshire, UK) was cut with the BamH1 restriction enzyme and dephosphorylated with calf intestinal alkaline phosphatase (CIAP) to prevent the vector from religating to

itself. The BamH1 cut pSVLGS.1 fragment was then ligated to a 2.4 kb BamH1 to Bgl2 fragment of pEE6hCMV (described in PCT Application No. WO89/01036, also available from Celltech) which was cut with Bgl2, BamH1 and Fsp1 to avoid two fragments of similar size, to yield an 11.2 kb vector designated p6/PSVLGS.1. pSVLGS.1 contains the glutamine synthetase selectable marker gene under control of the SV40 later promoter. The BamH1 to Bgl2 fragment of pEE6hCMV contains the human cytomegalovirus major immediate early promoter (hCMV), a polylinker, and the SV40 early polyadenylation signal. The coding sequences for soluble TNFR were added to p6/PSVLGS.1 by excising a Not1 to BamH1 fragment from the expression vector psolTNFR/CAVNOT (made according to Example 3 above), blunt ending with Klenow and ligating with SmaI cut dephosphorylated p6/PSVLGS.1, thereby placing the solTNFR coding sequences under the control of the hCMV promoter. This resulted in a single plasmid vector in which the SV40/GS and hCMB/solTNFR transcription units are transcribed in opposite directions. This vector was designated psolTNFR/P6/PSVLGS.

psolTNFR/P6/PSVLGS was used to transfect CHO-K1 cells (available from ATCC, Rochville, Md., under accession number CCL 61) as follows. A monolayer of CHO-K1 cells were grown to subconfluency in Minimum Essential Medium (MEM) 10 x (Gibco: 330-1581AJ) without glutamine and supplemented with 10% dialysed fetal bovine serum (Gibco: 220-6300AJ), 1 mM sodium pyruvate (Sigma), MEM non-essential amino acids (Gibco: 320-1140AG), 500 mu M asparagine and glutamate (Sigma) and nucleosides (30 mu M adenosine, guanosine, cytidine and uridine and 10 mu M thymidine)(Sigma).

Approximately 1 x 10<6 > cells per 10 cm petri dish were transfected with 10 ug of psolTNFR/P6/PSVLGS by standard calcium phosphate precipitation, substantially as described by Graham & van der Eb, Virology 52:456 (1983). Cells were subjected to glycerol shock (15% glycerol in serum-free culture medium for approximately 1.5 minutes) approximately 4 hours after transfection, substantially as described by Frost & Williams, Virology 91:39 (1978), and then washed with serum-free medium. One day later, transfected cells were fed with fresh selective medium containing MSX at a final concentration of 25 uM. Colonies of MSX-resistant surviving cells were visible within 3-4 weeks. Surviving colonies were transferred to 24-well plates and allowed to grow to confluency in selective medium. Conditioned medium from confluent wells were then assayed for soluble TNFR activity using standard binding assays. These assays indicated that the colonies expressed biologically active soluble TNFR.

In order to select for GS gene amplification, several MSX-resistant cell lines are transfected with psolTNFR/P6/PSVLGS and grown in various concentrations of MSX. For each cell line, approximately 1 x 10<6 > cells are plated in gradually increasing concentrations of 100 uM, 250 uM, 500 uM and 1 mM MSX and incubated for 10-14 days. After 12 days, colonies resistant to the higher levels of MSX appear. The surviving colonies are assayed for TNFR activity. Each of these highly resistant cell lines contains cells which arise from multiple independent amplification events. From these cells lines, one or more of the most highly resistant cells lines are isolated. The amplified cells with high production rates are then cloned by limiting dilution cloning. Mass cell cultures of the transfectants secrete active soluble TNFR.

Example 4

Effect of Soluble TNFR on Antigen-Induced Arthritis in Rats

Lewis rats previously immunized with methylated bovine serum albumin (mBSA) in complete Freund's adjuvant develop antigen-induced arthritis (AIA) when challenged with mBSA in knee joints. Administration of rhu TNFR:Fc, TNFR monomer, recombinant murine soluble IL-1 receptor (rm IL-1R) or a combination of TNFR monomer plus rm IL-1R was shown to be effective in suppressing the effects of antigen-induced arthritis in rats.

Lewis rats were immunized in the hind flank with 0.5 mg mBSA in complete Freund's adjuvant. Twenty-one days later (day 0), the animals were injected in both hind knee joints with 50 mu g mBSA in pyrogen-free saline. Groups of six rats were injected intra-articularly in both knee joints on that day and on the following 2 days (days 0, 1 and 2) as indicated below in Table A:

TABLE A
Treatment and Dosage Schedule

Group	Treatment	Dose
1	rhu TNFR/Fc	10 mu g
2	rhu TNFR/Fc	5 mu g
3	rmu IL-1 Receptor	1 mu g
4	TNFR Monomer	5 mu g
5	TNFR Monomer/rmu IL-1R	10 mu g/1 mu g
6	Diluent (saline)	-

Knee joint width was measured daily on days 0-6 relative to treatment. TNFR monomer was produced in CHO cells according to Example 2. The rhu TNFR:Fc used in this experiment was produced in BHK (hamster kidney) cells. This material is similar to the CHO cell-derived TNFR.

FIGS. 3 and 4 demonstrate that treatment with BHK-derived rhu TNFR:Fc at the time of mBSA challenge and for two days following challenge resulted in a reduction of knee-joint swelling in comparison to diluent-treated control rats. A reduction in joint swelling and inflammation was observed in rats treated with 5 or 10 mu g BHK-derived rhu TNFR:Fc or 5 mu g TNFR monomer or 1 mu g of rmuIL-1R. Reduction in joint swelling was even more pronounced when rmuIL-1R and TNFR monomer treatment was combined.

Histopathological examination of the joints harvested on day 6 was performed to confirm the degree of swelling. Histopathology scores were derived by evaluating knee joints and scoring their condition as follows: Grade 1, minimal, < 10% of area affected; Grade 2, moderate, 10-50% of area affected; Grade 3, marked, at least 50%, but less than all, of area affected; Grade 4, maximal, total area severely affected. A variety of lesions/alterations involving five knee joint structures were evaluated: joint capsule, joint space, synovial membrane, articular cartilage, and subchondral bone. Each structural alteration was scored from 1 to 4, and the scores were added and means were calculated. Histopathology results are expressed as the mean score in each treatment group.

The following Table B shows histopathology results, which also indicate that

rhu TNFR:Fc, TNFR monomer and rmu IL-1R were effective in reducing the severity of antigen-induced arthritis, and that a combination of rm IL-1R and TNFR monomer was more effective than either receptor alone.

TABLE B
Effect of rhu TNFR:Fc on Antigen Induced Arthritis in Rats

Treatment	Histopathology Score (Mean +/- SD (SE))	Number of Animals
Saline	18.4 +/- 4.9 (1.5)	10
1.0 mu g rmu IL-1R	13.1 +/- 4.7 (1.7)	8
10.0 mu g TNFR monomer	12.8 +/- 3.1 (1.1)	8
1.0 mu g rmu IL-1R/10.0 mu g TNFR monomer	7.9 +/- 5.2 (2.0)	5
5.0 mu g TNFR monomer	13.4 +/- 2.8 (1.0)	9
5.0 mu g rhu TNFR:Fc (BHK)	13.4 +/- 3.6 (1.3)	8

In summary, treatment with rhu TNFR/Fc, TNFR monomer, or rmu IL-1R at the time of mBSA challenge and for two days following challenge resulted in a reduction of knee-joint swelling in comparison to diluent-treated control rats. A combination of both rmu IL-1R and TNFR monomer resulted in greater reduction of swelling than either receptor molecule alone. Histopathology results also indicated that rhu TNFR/Fc, TNFR and rmu IL-1R were effective in reducing the severity of antigen-induced arthritis, and that a combination of rmu IL-1R and TNFR monomer was more effective than either receptor alone.

Example 5

Effect of Soluble TNFR on Collagen-Induced Arthritis in B 10.RIII Mice

B10.RIII mice previously immunized with porcine type II collagen (CII) in complete Freund's adjuvant consistently develop collagen-induced arthritis (CIA). Administration of rhu TNFR:Fc was shown to be effective in suppressing the symptoms of CIA in mice.

B10.RIII mice were immunized intradermally with 100 mu g porcine type II collagen (CII) in complete Freund's adjuvant to induced arthritic symptoms. Approximately 14-17 days post-immunization, symptoms of clinical arthritis began to appear in the mice, with 90-100% of the mice displaying severe arthritis by day 28. Mice were injected intraperitoneally with TNFR/Fc or PBS to determine the effect of soluble TNFR/Fc on CIA. Mice were assessed for symptoms of arthritis at 12 weeks post-immunization.

In a first experiment, TNFR/Fc was administered over the entire period of CIA development. Twelve mice were injected with 10 mu g TNFR/Fc, 3 days per week, from days 0 to 35. Twelve control mice were injected with PBS. FIG. 5 shows that TNFR/Fc significantly reduced the incidence of arthritis when compared to controls. Upon cessation of treatment with TNFR/Fc, the mice developed arthritis.

In a second experiment, TNFR/Fc was administered during only the developmental stages of CIA on days - 1-17 relative to immunization, as set forth in the following Table C.

TABLE C
Effect of rhu TNFR:Fc Administered During Inductive Stage of CIA

Treatment	Incidence (Positive/Total)	Onset (Mean Day +/- SE)	Severity (Mean +/- SE)
30 mu g TNFR/Fc Days - 1, 3	10/10	24 +/- 2	10.5 +/- 0.5
10 mu g TNFR/Fc Days - 1 to 17 (alternate days)	8/10	21 +/- 2	8.6 +/- 0.6
100 mu l PBS Days - 1 to 17 (alternate days)	10/10	18 +/- 1	10.6 +/- 0.4

These data show that TNFR/Fc delayed the onset of arthritis, but that CIA was unaltered in mice receiving 30 mu g TNFR/Fc the day before and 3 days after immunization with type II collagen. Mice given 10 mu g TNFR/Fc, every other day, from day - 1 to day 17 displayed a slight decrease in CIA incidence and severity versus controls injected with PBS.

In a third experiment, TNFR/Fc was administered during only the progressive stages of CIA every other day on days 14-28 post-immunization as set forth in the following Table D.

TABLE D
Effect of rhu TNFR:Fc Administered During Progressive Stage of CIA

Treatment	Incidence (Positive/Total)	Onset (Mean Day +/- SE)	Severity (Mean +/- SE)
10 mu g TNFR/Fc Days 14-28 (alternate days)	8/9	27 +/- 6	8.6 +/- 1.3
100 mu l PBS Days 14-28 (alternate days)	9/9	21 +/- 1	8.7 +/- 0.6

These data show that mice given 10 mu g TNFR/Fc, every other day, from days 14-28 showed a slight delay in CIA onset when compared to control animals. However, the incidence and severity of arthritis appears to be unaltered.

In summary, these experiments indicate that TNFR/Fc was effective in delaying the onset of CIA when administered over the entire course of CIA development.

Example 6

Effect of Soluble TNFR on Collagen-Induced Arthritis in DBA/1 Mice

The effect of soluble TNFR/Fc on CIA in DBA/1 mice previously immunized with porcine type II collagen (CII) in complete Freund's adjuvant was also tested. Administration of rhu TNFR:Fc was shown to be effective in suppressing the symptoms of CIA.

In this experiment, DBA/1 mice were immunized with 100 mu g of CII and then injected intraperitoneally with 50 mu g recombinant soluble human TNFR/Fc in sterile saline from day 21 to day 28. Control mice received sterile saline (vehicle) injections. This treatment period was prior to the development of the clinical signs of CIA, but during the development of DTH responses to type II collagen and rapid IgG anti-CII production.

Both groups of mice were assessed for the development of CIA for 70 days, and onset of CIA for 44-55 days post-immunization. FIGS. 6 and 7 show that TNFR/Fc significantly reduced the incidence of CIA compared with controls (28% vs. 86%; $p < 0.03$), and reduced both arthritis index (a subjective measure of severity) and the number of involved joints. The antibody response to CII was significantly lower immediately post treatment with TNFR/Fc (day 28), but antibody levels were equivalent at the conclusion of the experiment (day 70).

These results indicate that TNFR/Fc is effective in reducing the incidence of CIA in mice and may therefore be useful in the treatment arthritis.

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- (iii) NUMBER OF SEQUENCES: 5
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(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
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(G) CELL TYPE: Fibroblast
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(B) TYPE: amino acid
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CLAIMS: We claim:

[*1] 1. A method for lowering the levels of active TNF- alpha in a mammal in need thereof which comprises administering to said mammal a TNF-lowering amount of a TNF antagonist selected from the group consisting of:

(a) a TNF receptor comprising the sequence of amino acids 3-163 of SEQ ID NO:1; and

(b) a chimeric antibody comprising a TNF receptor according to (a) fused to the constant domain of an immunoglobulin molecule.

[*2] 2. A method for lowering the levels of active TNF- alpha in a mammal in need thereof which comprises administering to said mammal a TNF-lowering amount of a TNF receptor comprising the sequence of amino acids 3-163 of SEQ ID NO:1.

[*3] 3. A method for lowering the levels of active TNF- alpha in a mammal in need thereof which comprises administering to said mammal a TNF-lowering amount of a chimeric antibody comprising a TNF receptor comprising the sequence of amino acids 3-163 of SEQ ID NO:1 fused to the constant domain of an immunoglobulin molecule.

[*4] 4. A method for lowering the levels of active TNF- alpha in a mammal having arthritis, which comprises administering to such mammal a therapeutically effective amount of a TNF-antagonist selected from the group consisting of:

(a) a TNF receptor comprising the sequence of amino acids 3-163 of SEQ ID NO:1; and

(b) a chimeric antibody comprising a TNF receptor according to (a) fused to the constant domain of an immunogloblin molecule.

[*5] 5. A method for lowering the levels of active TNF- alpha in a mammal having arthritis, which comprises administering to said mammal a TNF-lowering amount of a TNF receptor comprising the sequence of amino acids 3-163 of SEQ ID NO:1.

[*6] 6. A method for lowering the levels of active TNF- alpha in a mammal having arthritis, which comprises administering to said mammal a TNF-lowering amount of a chimeric antibody comprising a TNF receptor comprising the sequence of amino acids 3-163 of SEQ ID NO:1 fused to the constant domain of an immunoglobulin molecule.

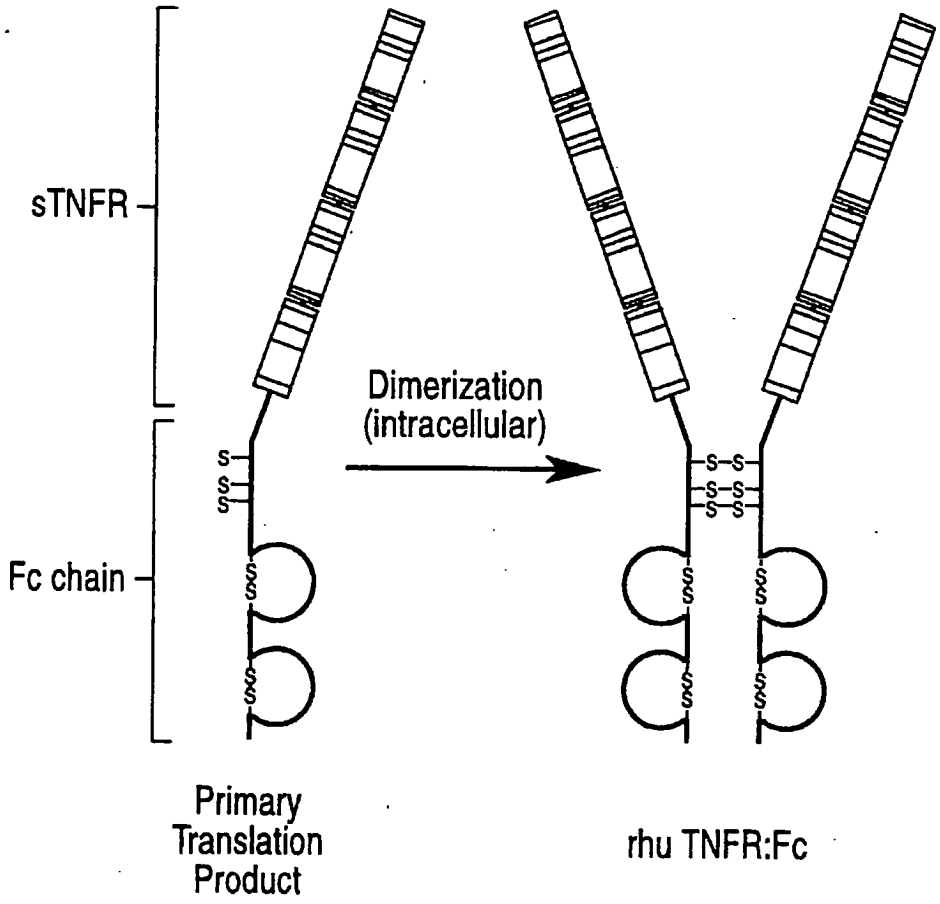
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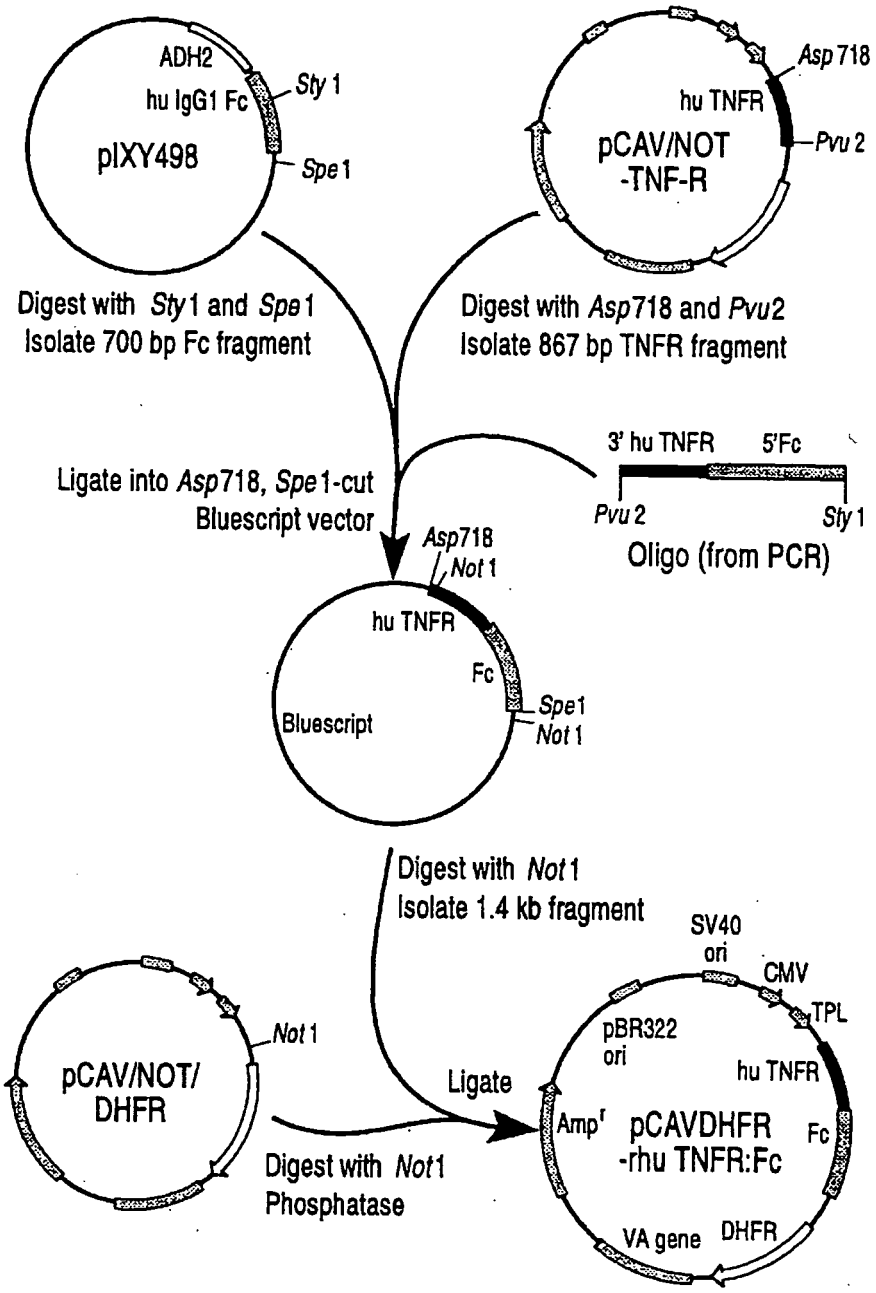
Figure 1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Pvu 2
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Fc
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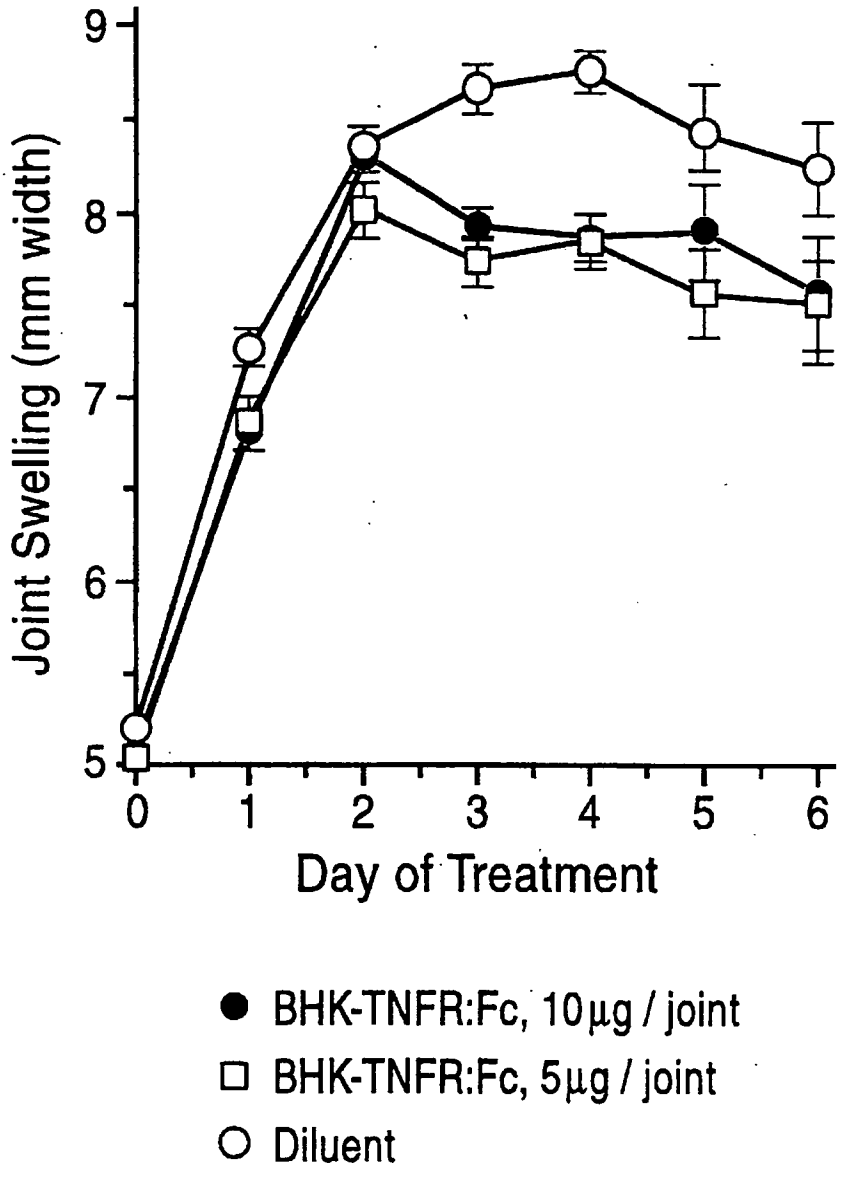
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8
7
6
5
0
1
2
3
4
5
6
Day of Treatment
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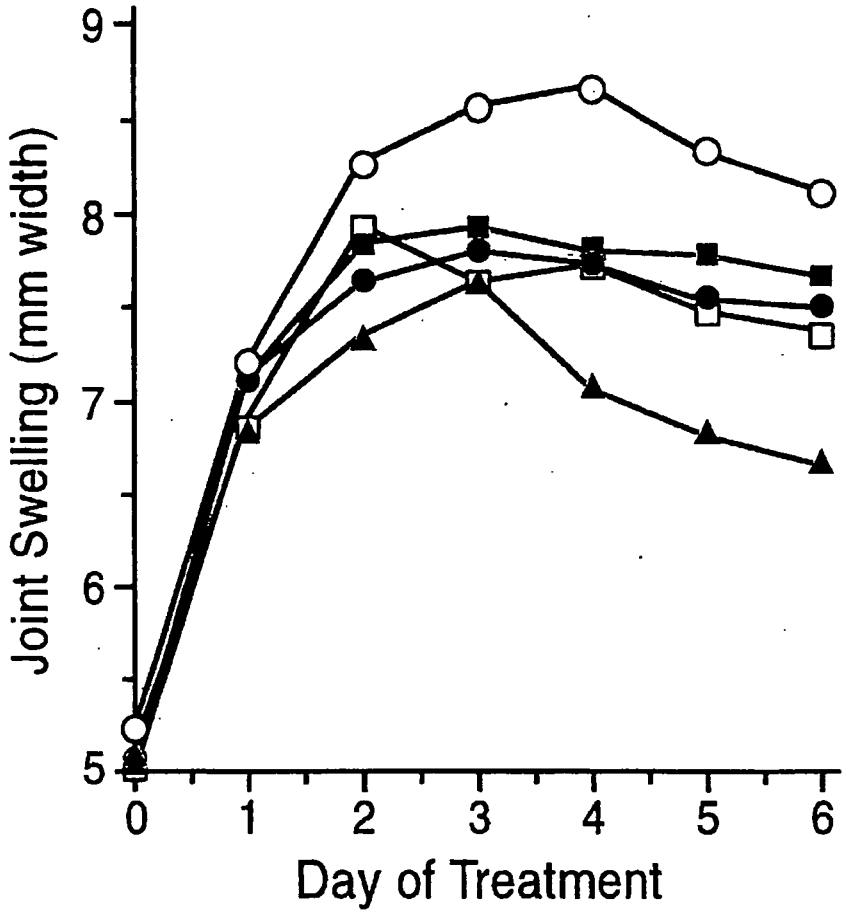
BHK-TNFR:Fc, 5µg / joint
Diluent

Figure 3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8
7
6
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1
2
3
4
5
6
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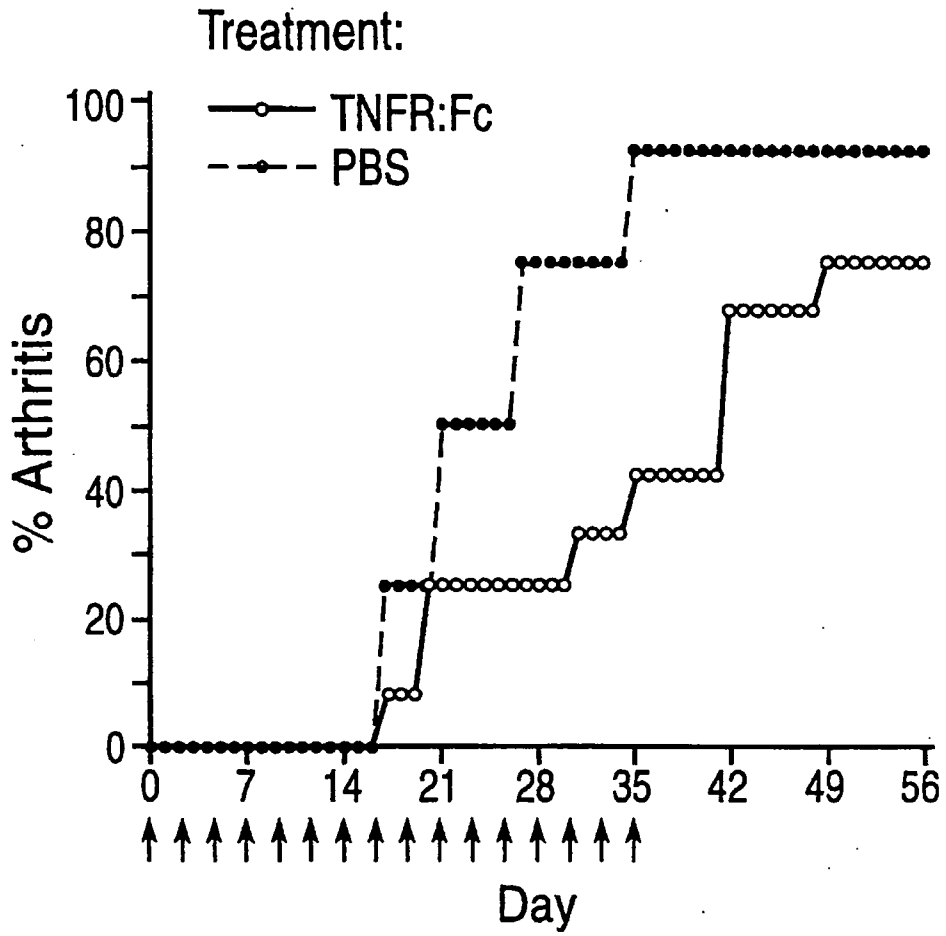
Diluent

Figure 4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21
28
35
42
49
56
Day

Figure 5

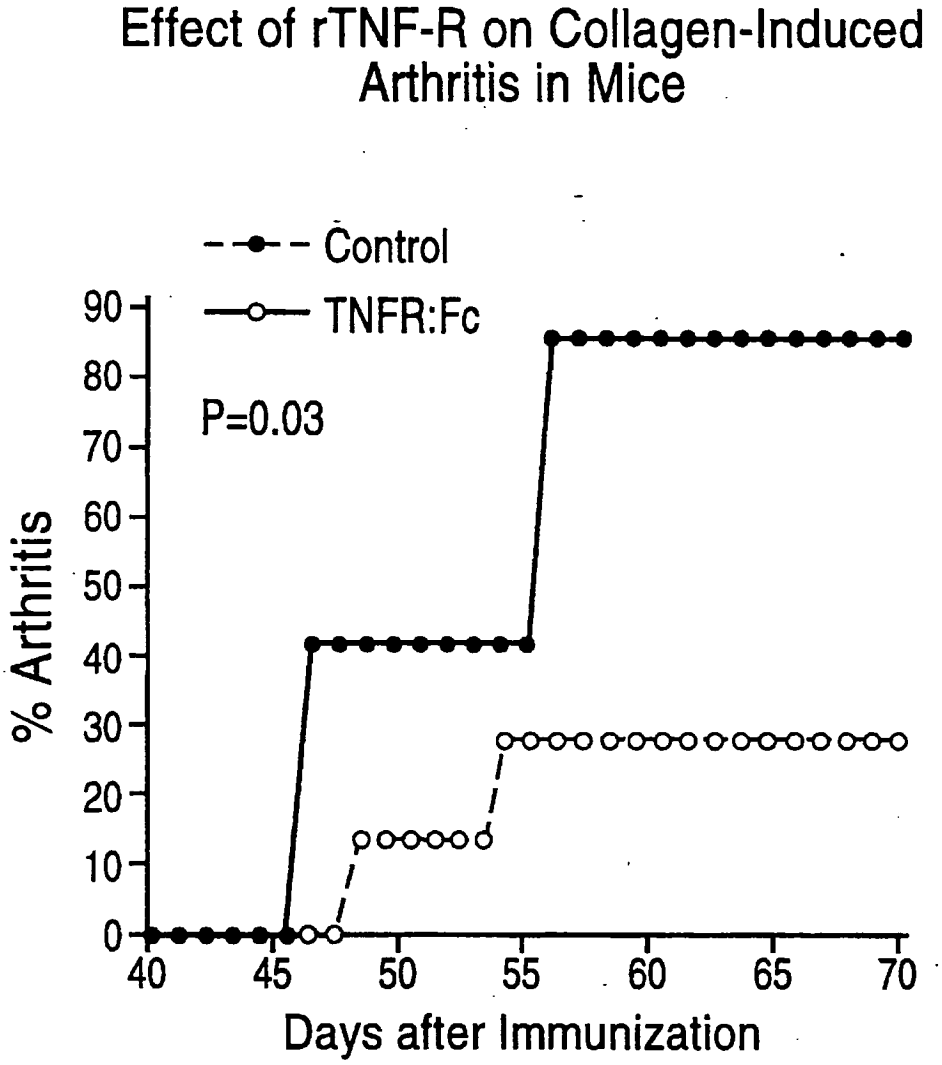

Figure 6

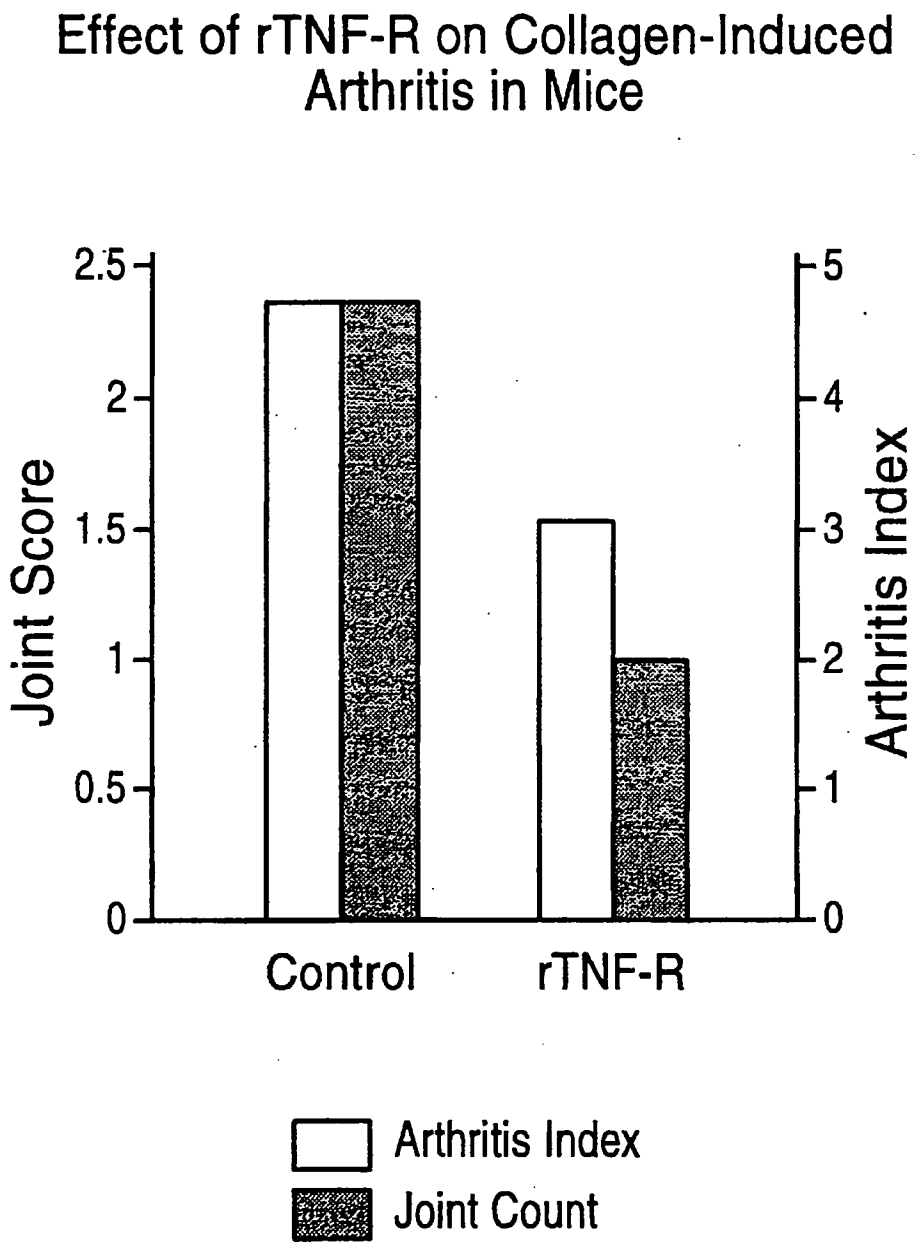
Effect of rTNF-R on Collagen-Induced Arthritis in Mice
Joint Score
2.5
2
1.5
1
0.5
0
Arthritis Index
5
4
3
2
1
0
Control
rTNF-R
Arthritis Index
Joint Count

Figure 7

1ST PATENT of Level 1 printed in FULL format.

TNF

5,795,967

Aug. 18, 1998

Tumor necrosis factor antagonists and their use

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APPL-NO: 482,226

FILED: Jun. 7, 1995

REL-US-DATA:
Continuation of Ser. No. 342,676, Nov. 21, 1994 now abandoned Which is a continuation of Ser. No. 174,212, Dec. 28, 1993 now abandoned Which is a continuation of Ser. No. 26,717, Mar. 5, 1993 now abandoned Which is a continuation of Ser. No. 707,412, May 28, 1991 now abandoned Which is a continuation of Ser. No. 417,171, Oct. 4, 1989 now abandoned Which is a continuation of Ser. No. 898,272, Aug. 20, 1986 now abandoned Which is a continuation-in-part of Ser. No. 754,507, Jul. 12, 1985 now abandoned And Ser. No. 881,311, Jul. 2, 1986 now abandoned Which is a continuation-in-part of Ser. No. 677,156, Dec. 3, 1984 now abandoned Which is a continuation-in-part of Ser. No. 627,959, Jul. 5, 1984 now abandoned

INT-CL: [6] C07K 16#00; C12N 5#06; A61K 39#395

US-CL: 530#388.23; 530#388.2; 530#388.4; 530#387.1; 530#866; 435#335; 424#133.1; 424#145.1

CL: 530;435;424

SEARCH-FLD: 424#130.1, 141.1, 133.1, 134.1, 139.1, 158.1; 530#387.1, 388.23, 388.2, 388.4, 387.9, 387.3, 866; 435#240.27

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PRIM-EXMR: Loring, Susan A.

LEGAL-REP: Marschang; Diane L.

ABST:
Tumor necrosis factor antagonists are administered in therapeutically effective doses to suppress inflammatory immune-potentiated events. The antagonists of this invention typically are selected from among several classes but preferably are neutralizing antibodies directed against tumor necrosis factor. The antagonists are useful in suppressing transplantation immunity and in the treatment of autoimmune diseases.

NO-OF-CLAIMS: 15

EXMPL-CLAIM: <=21> 1

NO-OF-FIGURES: 0

NO-DRWNG-PP: 0

PARCASE: This is a continuation of application(s) Ser. No. 08/342,676 filed on 21 Nov. 1994, now abandoned, which is a continuation application of Ser. No. 08/174,212 filed on 28 Dec. 1993, now abandoned, which is continuation application of Ser. No. 08/026,717 filed on 5 Mar. 1993, now abandoned, which is a continuation application of Ser. No. 07/707,412 filed on 28 May 1991, now abandoned, which is a continuation application of Ser. No. 07/417,171 filed on 4 Oct. 1989, now abandoned, which is a continuation application of Ser. No. 06/898,272 filed on 20 Aug. 1986, now abandoned, which is a continuation-in-part application of Ser. No. 06/754,507 filed on 12 Jul. 1985, now abandoned, and a continuation-in-part application of Ser. No. 06/881,311 filed on 2 Jul. 1986, now abandoned, which is a continuation-in-part application of Ser. No. 06/677,156 filed on 3 Dec. 1984, now abandoned, which is a continuation-in-part application of Ser. No. 06/627,959 filed on 5 Jul. 1984, now abandoned, which applications are incorporated herein by reference.

SUM:

This invention relates to the therapy of inflammatory, particularly immune-potentiated inflammatory events. In particular, it relates to the use of tumor necrosis factor antagonists for the suppression of graft rejections (of or by the host), and for the treatment of arthritis, systemic lupus, Crohn's disease and other autoimmune or inflammatory disorders.

The application of recombinant DNA techniques has resulted in the production of highly purified recombinant human tumor necrosis factor-alpha (rTNF- alpha) and -beta (rTNF- beta). These factors share amino acid sequence homology and show similarities in many of their biologic functions.

Tumor necrosis factors (TNFs) originally were identified by their ability to target tumor cells in vitro and in vivo for cytolysis or growth inhibition. Much of the interest attending the discoveries of these uses was based on their differential cytotoxicity. While TNFs will directly lyse many types of tumor cells, they have generally been considered to be relatively innocuous for normal untransformed, non-virally infected adult cells.

TNF- alpha has been said to have a central role in the immune response (Gamble et al., 1985, "Proc. Natl. Acad. Sci. USA" 82: 8667), but the nature of that role remains clouded. Both rHuTNF- alpha and beta activate human polymorphonuclear neutrophil functions in vitro (Shalaby et al., 1985, "J. Immunol." 135 : 2069-2073; Gamble et al., Id.) and rHuTNF- alpha modulates the function of endothelial cells in vitro (Stolpen et al., 1986, "Am. J. Pathol." 123: 16; Nawroth et al., 1986, "J. Exp. Med." 163: 740). See also Ezekowitz et al., "J. Clin. Invest." 76: 2368 (1985), Lommitzer et al., "Clin. Exp. Immunol." 29: 501 (1977), and Cross et al., "Infect. Immun." 22: 502 (1978). Also, recent studies showed the capacity of rHuTNF- alpha to act as an endogenous pyrogen and to induce interleukin-1 (IL-1) (Dinarello et al., 1986, "J. Exp. Med." 163: 1433 and Nawroth et al., 1986, "J. Exp. Med." 163: 1363).

On the other hand, Broxmeyer et al., "J. Immun." 136(2): 4487 (1986) reported that TNF- alpha and TNF (LuKII) suppress in vitro colony formation by human bone marrow granulocyte-macrophage, erythroid and multipotential progenitor cells. The immunosuppressive effect of TNF (LuKII) and TNF- alpha was inhibited, respectively, by polyclonal anti-human TNF(LuKII) and monoclonal anti-recombinant human TNF- alpha . Similarly, Murphy et al. suggested that

activated T cells regulate hematopoiesis through the release of inhibitory as well as stimulatory factors, and that the simultaneous production of IFN- gamma and lymphotoxin (TNF- alpha) may represent a mechanism of suppression of hematopoiesis ("J. Exp. Med." 164: 263, July 1986). See also Degliantoni et al., "J. Exp. Med." 162: 1512 (1985). Fujisawa et al. have reported that immunosuppression by monocytes from patients with tuberculosis is associated with increased production of IL-1 ("Am. Rev. Respir. Dis." 133: 73 (1986), and it has been believed in certain quarters that IL-1 is a mediator of immunosuppression (Wallis et al.). In any case, little is known that would correlate the in vitro effects of HuTNF- alpha and - beta to the roles of these factors in the complex matrix of the immune inflammatory response in vivo.

Transplantation immunology has advanced on two fronts. In one respect, research has been directed towards the development of host immunosuppressive drugs such as cyclosporin antibiotics and steroids. Such drugs exert extensive and undesirable side effects. Other research has concentrated on the use of monoclonal antibodies to target lymphocyte subsets involved in transplant rejection. Since antibodies are directed against an entire lymphocyte subset they are not focused on any principal mediator of immune inflammatory response. Their activity, like that of cyclosporins and steroids, therefore, is relatively nonspecific. It would be best if immunosuppressive agents were targeted against an effector or mediator rather than an entire cell subset since the subset may be involved in desirable mechanisms of the immune response.

A substance(s) identified as "lymphotoxin" has been postulated to be involved in or to be a mediator of delayed type hypersensitivity. For example, "lymphotoxin" is released by the peripheral blood lymphocytes of polymyositis patients when incubated in vitro with autochthonous muscle tissue in vitro (Johnson et al., 1972 "J. Clin. Invest." 51: 2435) and has been found in rejecting allografts (Lowry et al., 1985, "Transplantation" 40(2): 183-188). It has been postulated that "lymphotoxin" and gamma interferon may have synergistic deleterious effects on the integrity of transplanted tissues. However, it also was recognized that other potent macromolecular toxins are released by NK cells, cytotoxic T cells and macrophages, and their potential role in delayed type hypersensitivity and tissue injury remain to be elucidated (Lowry et al., Id.)

"Lymphotoxin" also was reported to be released by sensitized T lymphocytes from myocardial infarct patients exhibiting delayed type hypersensitivity to myoglobin (Mirrakhimov et al., 1985, "Ter Arkh." 56(10): 53-56).

"Lymphotoxin" has been postulated to be induced in abnormally high quantities in infected T cells by the trans acting protein of human immunodeficiency virus. These abnormally high quantities are proposed to result in immunosuppression and T cell self-destruction (Ruddle, 1986, "Immunology Today" 7: 8-9).

Korsmeyer et al., "Clin. Immun. and Immunopathology" 5: 67-73 (1976) disclosed data consistent with the hypothesis that naturally occurring anti-lymphocyte antibodies in systemic lupus erythematosus (SLE) are a secondary manifestation of a suppressor cell defect with possible specificity for nonsuppressor T-cell subpopulations such as helper or killer cells, a view consistent with the report that anti-thymocyte antibodies were capable of limiting allograft rejection (Gelford et al., 1974, "J. Immunol. 113:1) and thus may be more specific for helper or killer cell functions. See also Terasaki et al., "New Engl. J. Med." 283(14): 724 (1970) wherein anti-lymphocytic antibodies

in the sera of SLE and arthritis patients were referred to as "lymphotoxin". For the role of lymphocytotoxic antibodies in inflammatory bowel disease see Strickland et al., "Clin. Exp. Immunol." 21: 384-394 (1975).

Two classes of nonacidic drugs used to treat arthritis and other inflammatory diseases were found to inhibit lysis of murine L cells by PHA-P induced human lymphocyte cytotoxins, but the acidic anti-inflammatory compounds salicylate and phenylbutazone gave little protection against the cytotoxin (Peter, 1971, "Cell. Immunol." 2: 199-202). The cytotoxin(s) were characterized as having an approximate molecular weight of 80,000 d. In this regard see Peter et al., "Arthritis Rheum." 14:179 (1971), who report the isolation of cytotoxins from synovial fluid. It is difficult to square any postulatable role for lymphocyte cytotoxins in view of recent reports that gamma-interferon ameliorates arthritis in cancer patients. Gamma-interferon is known to act synergistically with TNF- beta and TNF- alpha in antiviral and antiproliferative assays.

Other quandaries exist in the arthritis field. For example, research has been directed at drugs that block interleukin-1, presumably in order to suppress immune function, while other workers have suggested administering interleukin-2, despite its immunopotentiating action, because of indications that arthritis patients are deficient in interleukin-2 ("Wall Street Journal", Jul. 28, 1986).

In summary, a variety of tentative and hypothetical postulates exist for the mechanisms underlying various. immune-mediated inflammatory responses. Many of the postulates are mutually inconsistent and most are based on observations which cannot distinguish cause from epiphenomenology.

Accordingly, it is an object herein to provide compositions that are in fact capable of precisely targeting acute immune inflammatory responses without producing significant undesirable side effects.

This and other objects will be apparent from consideration of the specification as a whole.

SUMMARY

The objects of this invention are-accomplished by a method comprising administering to a patient with an inflammatory condition a therapeutically effective amount of an antagonist for TNF- alpha and/or TNF- beta . It is not necessary to administer an interferon antagonist, nor to use other immunosuppressive substances in the course of therapy. The method herein is particularly useful in suppressing graft versus host and host versus graft rejections in organ transplants, the former including in particular bone marrow transplants. Preferably, the antagonist is a TNF- alpha antagonist.

DETAILED DESCRIPTION

For convenience, the term TNF collectively shall mean tumor necrosis factor- alpha or - beta from animals or humans, together with naturally occurring alleles thereof. TNF- alpha is described by Pennica et al. "Nature" 312: 721 (1984). TNF- beta , also referred to as lymphotoxin, is described by Gray et al. "Nature" 12: 724 (1984).

The novel compositions for use herein are TNF antagonists. These substances function in one of two ways. First, antagonists will bind to or sequester the TNF molecule itself with sufficient affinity and specificity to substantially neutralize the TNF epitopes responsible for TNF receptor binding (hereinafter termed sequestering antagonists). Alternatively, TNF antagonists will compete with native TNF for the cell surface receptor or intracellular site to which TNF binds in the course of cell lysis (hereinafter termed competitive antagonists). Both groups of antagonists are useful, either alone or together, in the therapy of immunoinflammatory responses.

Sequestering antagonists include TNF cell surface receptors and neutralizing antibodies to TNF. TNF- alpha neutralizing antibodies are readily raised in animals such as rabbits or mice by immunization with TNF- alpha in Freund's adjuvant followed by boosters as required. Immunized mice are particularly useful for providing sources of B cells for the manufacture of hybridomas, which in turn are cultured to produce large quantities of inexpensive anti-TNF- alpha monoclonal antibodies. Surprisingly, murine monoclonal antibodies have been obtained that exhibit high anti-TNF- alpha activity, on the order of 10<10 > liters/mole. TNF- beta neutralizing antibodies are prepared by the method described in European patent application 168,214.

TNF receptors are obtained by first identifying a cell line obtained from the species to be treated with the antagonist that is cytolysed by TNF. Examples of suitable receptor sources include ME-180 and, for TNF- alpha receptors only, placenta. The cells are cultured (if cell lines), lysed, cell membranes recovered by centrifugation, the membranes extracted with detergent (preferably Triton X-100) and the protein in the membrane extract dialyzed free of detergent. Since both TNF- alpha and TNF- beta are capable of binding a common receptor, this receptor may be purified by affinity binding with either TNF- alpha or TNF- beta and is suitable for neutralizing either factor. Preferably, the receptors are further purified by immunoprecipitation with murine neutralizing monoclonal antibody, rabbit anti-mouse antibody and polyethylene glycol, followed by redissolution and separation on nondenaturing gel electrophoresis.

The receptor preparations also are useful for immunizing animals in conventional fashion to raise anti-receptor antibodies that are capable of inhibiting the binding of TNF to its receptor. These will be screened for in the same routine fashion as are TNF competitive antagonists.

Monoclonal and polyclonal anti-TNF antibodies suitable for use herein may vary widely in their affinity for TNF and their ability to neutralize TNF. Deficiencies in affinity or capacity on the part of the antibody can be made up by dosage increases. Acceptable polyclonal antibody should be capable of neutralizing greater than about 400 TNF units/ mu l, ordinarily greater than about 900 units (with TNF containing about 10<8 > units/mg of purified TNF). Polyclonal rabbit antisera will neutralize about 900 units/ml. Murine monoclonal anti-TNF- alpha antibodies have been obtained that neutralize 2700 and 2300 units of TNF- alpha / mu g, respectively, when purified by conventional methods using ammonium sulfate fractionation followed by DEAE sepharose column chromatography. Two neutralizing epitopes for TNF- alpha have been identified. One of these, the D/E epitope is the most prevalent by far. The other epitope, the B epitope, is quite rare.

Polyclonal antisera from immunized animals or monoclonal antibodies in hybridoma culture are harvested by techniques known per se and purified to the desired degree. Typically, the immunoglobulin fraction is separated from the antisera or hybridoma culture supernatants by conventional methods, for example ethanol or polyethylene glycol fractionation. Preferably the antibodies are of the IgG class, although other classes such as IgM are acceptable. Further, the anti-TNF antibodies alternatively are provided as monovalent subunits such as Fab fragments containing the TNF-binding variable region. Such subunits will exhibit less side effects, e.g. immunogenicity, than intact antibodies containing constant regions. Methods are well-known per se for the preparation of Fab fragments from intact antibodies.

Competitive TNF antagonists preferably are TNF antagonistic variants. Such variants include substitutions, deletions or insertions of residues (amino acid sequence variants) as well as other covalent modifications, e.g. alkylated TNF. However, amino acid sequence variants are most preferred as they are readily manufactured in recombinant cell culture and recovered as chemically uniform, homogeneous compositions. Further, covalent modifications are more likely to immunize the patient against TNF, an event that is undesirable from the standpoint of long term patient recovery.

Antagonistic TNF amino acid sequence variants are variants of the mature TNF amino acid sequence that are capable of inhibiting TNF cytotoxic activity, but which have substantially no cytotoxic activity of their own. Antagonistic TNF sequence variants will competitively bind to cell surface receptors or intracellular TNF recognition sites without exerting any substantial cytotoxic effect, thereby displacing TNF or preventing TNF from binding to or interacting with the cells.

Antagonistic TNF sequence variants are characterized by amino acid deletions, substitutions and/or insertions which lead to the substantial inactivation of TNF cytotoxic activity without substantially interfering with the ability of the antagonist to inhibit TNF cytotoxic activity. Typically, deletional mutations are preferred as they are less likely to induce an active anti-TNF immune response in patients to whom the antagonists are administered.

The sites within the human TNF- alpha molecule that are selected for sequence variation generally are located within about residues 10 to 66, 113 to 134 and 150 to 157 (numbered following Pennica et al., Id.) Residues within these regions are believed to be important for TNF- alpha cytotoxic activity. Since an amino terminal domain within about residues 1 to 40 is believed to be externalized and therefore likely to be involved in receptor binding, non-cytotoxic analogues that are capable of competing with endogenous TNF- alpha generally will be mutated at points in the C-terminal direction from this region. Deletions, substitutions or insertions of disparate residues in these regions (40-66, 110-134 and 150-157) are anticipated to yield TNF variants having reduced or no cytotoxic activity combined with varying degrees of antagonist activity. Those mutants having the optimum combination of antagonist activity and reduced cytotoxicity are identified by the screening assays described above.

Sites within the human TNF- beta molecule that are preferred for mutation generally are located within about residues 28-37, 46-55, 65-81, 136-149 and

166-171 (numbered following Gray et al., Id.). More detailed specific embodiments are set forth below.

In the process of making TNF amino acid sequence variants, existing residues in the native TNF molecule will be substituted by or flanked by inserted residues that are unlike those found at the site of substitution or insertion. Residues characterized as unlike are those for example which bear a substantially different charge or affinity for water than the residue which is substituted or adjacent to which an insertion is made. For example, amino acid residues in the following groups are substituted for one another or inserted adjacent to residues from different groups. Hydrophobic residues are methionyl, phenylalanyl, glycyl, valyl, leucyl and isoleucyl. Hydrophilic, uncharged amino acids include tyrosinyl, seryl and threonyl. Negatively charged residues include glutamyl or aspartyl, while basic residues are represented by lysyl, hydroxylysyl, arginyl and histidyl. Heterocyclic residues are tryptophanyl, prolyl and hydroxyprolyl. In accordance with this invention, residues from any one of these groups are substituted for or inserted adjacent to a residue from any other group in order to produce candidate variants.

TNF antagonists are easily identified and rated by routine screening of candidates for their effect on the activity of native TNF on susceptible cell lines in vitro. For example, the conventional L-cell assay for TNF activity is employed with a standard cell-lytic TNF formulation containing varying dilutions of candidate antagonist, e.g. from 0.1 to 1000 times the molar amount of TNF used in the assay, and controls with no TNF or no-antagonist. The amount of TNF activity, in units, neutralized by the antagonist candidate will be recorded as the antagonizing titer in vitro.

In more detail, TNF antagonists are identified as follows. First, the candidate substances are screened for TNF antagonist activity in a modification of a standard in vitro TNF cytotoxicity assay (Aggarwal et al., 1984, Thymic Hormones & Lymphokines. Goldstein. Ed. Plenum Publishing. p. 235). Target cells, generally a publicly available TNF-susceptible murine L-929 cell line or a susceptible human cell line, are seeded into microtiter wells and grown to confluence. Serial dilutions in culture medium of the candidate together with a standard cytotoxic quantity of TNF are incubated at 37o C. for 16 hrs. and then pipetted into the wells. The cells are incubated to the point that the TNF controls demonstrate cytotoxic effect on at least 50% of the cells. Thereafter, the test wells are observed and scored for the proportion of protected cells. This is a measure of the ability of the candidate to block TNF cytotoxicity. TNF cytotoxicity is identified for example by failure of treated cells to take up stain (crystal violet) or by their release of an isotope such as Cr<51> . Large numbers of candidate substances and dilutions are readily screened by automatic equipment, e.g. the Microelisa autoreader (Dynatech). Controls containing candidate alone are useful for determining the residual cytotoxic activity of competitive-type antagonists.

An initial in vivo screening procedure is recommended whereby the TNF antagonists identified in the in vitro assay are assayed for the same activity in an in vivo context. The preferred assay is a modification of the Meth A sarcoma model of E. Carswell et al., "Proc. Nat. Acad. Sci." 72:3666-3670 (1975). Briefly, this modified assay is carried out by growing Meth A sarcoma cells (5 x 10<5 > cells) in CB6F1 female mice (BALB/c x C57BL/6)F1 for 7-10 days. The tumors that develop are injected with serial dilutions of a

preincubated solution containing TNF and a candidate substance as described above. After 24 hours, mice are sacrificed by cervical dislocation, tumors removed and necrosis scored histologically. Candidates that reduce or inhibit the necrotic activity of TNF on the sarcomas are selected for further screening.

The first step in the screening procedure (the in vitro cell culture screen) can be omitted but the efficiency of identifying effective antagonists will be lowered correspondingly. The third step in the TNF antagonist screening procedure comprises determining the activity of the antagonist in a known animal model. For example, serial dilutions of the candidate are administered to mice prior to or at points in time after bone marrow transplantation, after which suppression of the graft versus and the host rejection by the transplanted marrow monitored, for example by following splenomegaly. This is useful in confirming antagonist activity in vivo as well as for determining suitable dosages. Alternatively, the murine collagen-induced arthritis model is suitable for screening activity and determining effective doses for each candidate.

Inflammatory or immune-potentiated inflammatory events to be treated with TNF antagonists are characterized by the presence of a humoral and/or cellular response directed against an undesired foreign or self target tissue or by uncertain etiology. Typically, immune potentiating inflammatory events are characterized by antibodies directed against host tissue by way of an aberrant host response, host antibodies against grafted tissue, or antibodies of graft origin directed against host tissue. Such events also are characterized by infiltration of polymorphonuclear neutrophils and mononuclear leukocytes into the target tissue, pain, localized edema, possible vascular endothelial injury and excessive production of cytokines by stimulated cells. Other than in transplantation immunity, such events occur during the course of diseases including arthritis, systemic lupus, Crohn's disease, and other autoimmune disorders known to those skilled in the art, and frequently in inflammatory conditions such as sarcoidosis or vasculitis.

The therapeutically effective amounts of TNF antagonist will be a function of many variables, including the type of antagonist, e.g. whether TNF sequestering or competitive, the affinity of the antagonist for TNF, any residual cytotoxic activity exhibited by competitive antagonists, the route of administration, the clinical condition of the patient (including the desirability of maintaining a non-toxic level of endogenous TNF activity), the presence of multiple TNF combining sites in sequestering agents, e.g. antibodies, and whether or not the antagonist is to be used for the prophylaxis or for the treatment of acute rejection episodes. Since the maximum tolerated dose of TNF- alpha in human clinical trials has ranged up to about 25 mu g/m<2> /24 hrs, the amount of antagonist administered generally need not exceed a dose which is calculated to neutralize this amount of TNF- alpha . Accordingly, the molar dose of TNF antagonist will vary about from 0.001 to 10 times the maximum tolerated molar dose of TNF- alpha , although as noted above this will be subject to a great deal of therapeutic discretion. It is to be expected that concentrations of TNF localized at the sites of inflammation may exceed the whole body maximum therapeutic dose. Assay of the TNF concentration in inflammatory infiltrates will provide guidance as to the amount of TNF antagonist to be employed, particularly if localized administration is practical, e.g. in Crohn's disease (suppositories) or arthritis (injections into synovial fluid). Similar dosages and considerations apply in the case of TNF- beta . The key factor in selecting an appropriate dose is the result obtained: If the patient's inflammatory

response does not at least partially resolve within about 48 hours after administration, the dose should be gradually elevated until the desired effect is achieved. Correspondingly higher doses of polyclonal anti-TNF having a lower titer of TNF neutralizing activity and/or lower affinity for TNF will be required, and vice versa for antibody preparations with greater affinity and titer. Also, relatively higher doses will be initially needed for the treatment for acute rejection or inflammatory episodes, i.e., for patients in acute organ transplant rejection or undergoing arthritic flares.

In view of the therapeutic urgency attendant acute rejection episodes, the TNF antagonist should be intravenously infused or introduced at the inflammatory lesion immediately upon the development of symptoms or serological evidence of TNF activity. However, prophylaxis is suitably accomplished by intramuscular or subcutaneous administration.

The TNF antagonist is administered in conjunction with other anti-inflammatory agents used in or proposed for the treatment of individual immunoinflammatory conditions as appropriate, e.g. gold colloids, cyclosporin antibiotics, salicylate and corticosteroids (such as methylprednisolone). However, when employed together with TNF antagonists these agents may be employed in lesser dosages than when used alone.

The following examples are to be construed as merely illustrative of the invention and not as limiting its scope. All citations herein are expressly incorporated by reference.

DETDESC:

EXAMPLE 1

Preparation of Neutralizing Antibody to Human TNF- alpha

Human TNF- alpha was synthesized in recombinant culture by the method of Pennica et al. (op cit.) or isolated from cultured induced HL60 cells or peripheral blood lymphocytes. A BALB/c mouse (#185) was injected with human TNF-alpha immunogens on the following immunization schedule:

Day	Administration Route	Immunogen
1.	subcutaneous (sc) * * *	0.5. ml of 30,000 units TNF- alpha from human peripheral blood lymphocytes in PBS + 0.5 ml Freund's complete adjuvant.
	intraperitoneal (ip) *	0.5 ml of 30,000 units TNF- alpha from peripheral blood lymphocytes in PBS.
15.	half sc., half intramuscular (im)	5 x 10<4 > units TNF- alpha on 1.64% alum
43.	half sc., half im. *	10 mg E. coli recombinant human TNF- alpha on 0.1 ml 1.64% alum.
49.	intravenous (iv)	50 mu l of 1.3 mg/ml recombinant TNF- alpha .
74.	sc *	50 mu l of 2.5 mg/ml recombinant TNF- alpha + 100 mu l Freund's complete adjuvant.
81.	iv	2 mu g recombinant TNF- alpha .

84.	ip	200 mu g recombinant TNF- alpha .
85.	ip	400 mu g recombinant TNF- alpha (10 mu g iv).
86.	ip	400 mu g recombinant TNF- alpha (10 mu g iv).
87	ip	400 mu g recombinant TNF- alpha .

The anti-TNF- alpha neutralizing titer increased only gradually throughout the immunizations to day 74. The "Barragen" procedure conducted on days 81-87 ("J. Imm. Meth." 32: 297-304, 1980) reduced serum titer but increased the population of antigen-reactive cells, thereby greatly increasing the efficiency of the subsequent fusion procedure in producing useful clones. Retrospectively, only several alum-TNF- alpha immunizations are believed to be needed before the Barrage procedure in order to obtain satisfactory titers.

On day 88 the spleen from mouse 185 was harvested, disrupted and the spleen cells fused with P 3 x 63-Ag8.653 (ATCC CRL 1580) cells using the PEG fusion procedure of S. Fazekas de St. Groth et al., "J. Imm. Meth." 35: 1-21 (1980). The fused culture was seeded into 480 microtiter wells and cultured in conventional manner. The anti-TNF- alpha activity of culture supernatants was determined by adding a sample of the supernatant to microtiter wells precoated with recombinant human TNF- alpha , incubated, washed, horseradish peroxidase labelled-goat anti-mouse IgG added to the wells, incubated, washed and the bound HRP activity determined. 51 of the 480 wells contained clones making anti-TNF- alpha . Of these, 14 stable fusions which secreted anti-TNF- alpha were selected.

IgG purified from anti-TNF- alpha containing culture supernatants by ammonium sulfate precipitation and DEAE column chromatography was mixed with a solution of TNF- alpha in PBS, and incubated for 16 hours at 37o C. in order to ensure maximal binding. The incubation mixture was then assayed using the L cell cytotoxicity assay (Aggarwal et al., infra). The neutralizing titer is expressed as the number of TNF units in the cytotoxicity assay that are neutralized per mu l of unpurified antiserum or hybridoma supernatant culture medium. 10 cultures of the 14 produced neutralizing antibodies. Of the 10, TNF- alpha D (IgG1) exhibited an affinity of 10<10 > liters/mole and neutralizing titer of 2700 units/ mu g, TNF- alpha E (IgG1), 10<10 > liters/mole and a titer of 2300 units/ mu g and TNF- alpha B, 10<9 > liters/mole and per mu g neutralized 80% of 4074 TNF- alpha units. The D and E types are apparently directed at substantially the same TNF- alpha epitope because they compete for TNF- alpha binding. The B type competes at about the 50% level for the D/E epitope, thus suggesting that it is specific for its own unique epitope. TNF- alpha B was obtained from a single hybridoma (Genentech deposit no. 15-3-5E3H3).

Rabbit polyclonal antisera were obtained by immunizing New Zealand white rabbits with 0.6 mg TNF- alpha /0.5 ml PBS + 1.5 ml of Freund's Complete adjuvant (FCA) sc on day 1, 125 mu g TNF- alpha /0.125 ml PBS + 375 mu g FCA sc on day 36 and 125 mu g TNF- alpha /0.5 ml PBS + 1.5 ml FCA sc about 2 months later. Antisera harvested after the final booster contained a neutralizing titer of about 900 units/ mu l.

EXAMPLE 2

Preparation of TNF- alpha and TNF- beta Antagonist Analogues

The following M13 mutagenesis method, based on J. Adelman et al., "DNA" 2(3): 183-193, is generally applicable to the construction and expression of any TNF DNA sequences encoding antagonist TNF sequence variants. Additional information relating to M13 mutagenesis is provided by U.K. patent application 2,130,219A. Other methods suitable for creating TNF analogues are known to those in the art. For example, mutant DNA is constructed by simply chemically synthesizing the entire sequence or by synthesizing a portion of the sequence and ligating the fragment into the remainder of the required DNA. Chemical DNA synthesis is advantageous when the artisan wishes to prepare the mutant directly without first obtaining from natural sources the DNA encoding tumor necrosis factor. Ordinarily, however, the starting DNA will encode the natural amino acid sequence, including its allelic variants.

In order to avoid redundancy, representative contemplated sequence variants of TNF- alpha are illustrated in which Trp 28 or 114 is converted to phenylalanine or tyrosine (substitutions), Val 150 through Leu 157 or Lys 11 through Arg 31, inclusive, is deleted (deletions) and Ser-Ser-Ser is inserted after Phe 152 or Leu 26 (insertions). Other TNF- alpha variants which are representative candidates include DELTA ala4 his15, DELTA leu55 tyr56, tyr59 arrow right ile, gln61 arrow right trp, gly121 arrow right pro, phe124 arrow right ser, gly129 arrow right pro, arg131 arrow right asp, leu132 arrow right trp, and ala134 arrow right tyr. It should be understood, however, that other variants of TNF- alpha or TNF- beta are generated in the same general fashion.

Contemplated sequence variants of TNF- beta fall within residues 28-37, 46-55, 65-81, 136-149 and 166-171 (numbered as in Gray et al., Id.). Preferably, only one of the sequences 28-34, 51-54, 65-81 and 143-149 is varied. For example, representative embodiments include DELTA lys28-his32, DELTA arg51-leu54, DELTA leu65-pro68, DELTA ile72-val75, DELTA val75-val79, DELTA gln78-phe81, DELTA ala137-gln[140], DELTA asp130-ser148, lys28 arrow right thr, lys28 arrow right asp, ala30 arrow right lys, ala31 arrow right asp, his32 arrow right tyr, arg50 arrow right glu, ala51 arrow right tyr, phe53 arrow right lys, leu54 arrow right his, leu66 arrow right tyr, tyr73 arrow right lys, tyr76 arrow right lys, gln78 arrow right tyr, phe81 arrow right ser, gln[140] arrow right tyr, leu141 arrow right trp, asp130 arrow right phe, gln131 arrow right leu, ser148 arrow right asp, ala170 arrow right tyr, pro29 pro ala30, tyr73 tyr phe74, ser77 ser gln78, and asp145 asp gln146. Each substitution or variation at a given site in TNF- alpha may be made in TNF- beta , and vice versa, at the corresponding residue as shown in FIG. 4 of Pennica et al., Id. In addition, other substitutions at any of the representative sites set forth above are suitable for generating candidate variants.

Identification of operative and optimal embodiments is straight-forward. Routinely, one dilutes preparations of each variant in a constant amount of TNF-alpha or TNF- beta having cytotoxic activity and then assays the formulations in the conventional L-929 assay, screening for a reduction cell lytic activity upon an increase in relative proportion of candidate variant.

It will be appreciated that even if a candidate antagonist fails to exert antagonistic activity it will remain useful as an antitumor agent if it retains cytotoxic activity, or as a standard or labelled reagent for immunoassay of

native TNF so long as it retains at least one cross-reacting immune epitope.

It is desirable in preparing DNA encoding variant TNF derivatives that no codon changes be made which create the opportunity for mRNA transcribed therefrom to form high energy (arithmetically greater than about - 15 kcal/mole) stem-and-loop structures. Avoidance of DNA that is transcribed into mRNA containing such structures will generally result in higher yields. In addition, transformant host-preferred codons should be employed to enhance translational efficiency.

Suitable starting DNA for TNF- alpha variants is the EcoRI-HindIII fragment of pTNFTrp (Pennica et al., op cit.) obtained by sequential digestion with EcoRI and HindIII, followed by isolation of the TNF- alpha gene-containing fragment. This fragment includes the trp promoter and structural gene for methionyl-tumor necrosis factor- alpha . To obtain a single-stranded copy of this gene suitable for mutagenesis, the EcoRI-HindIII fragment is cloned into the polylinker site of phage M13 mp8 RF-DNA (J. Messing et al., 1982, "Gene" 19: 269-276; "RF" means the replicative form of the phage; this phage is commercially available). An aliquot of the EcoRI-HindIII digestion mixture is added to a ligation reaction containing 10 ng of M13 mp8 RF-DNA which had been digested previously with EcoRI and HindIII. After incubation at room temperature for 2 hrs, the ligation mixture is used to transform E. coli JM103 (a commercially available strain; JM101 can also be used). Transformed cells are plated with top agar containing X-GAL (dibromo-dichloro-indolyl-galactoside) and IPTG (isopropylthiogalactoside). Bacterial cultures (1 ml) infected with phage picked from colorless plaques are used to isolate M13 mp8/TNF RF-DNA by a miniscreen procedure (Birnboim and Doly, 1980, "Nuc. Acids Res." 7:1513-1523). The resulting recombinant phage M13 mp8/TNF carries the coding strand for the TNF-alpha gene.

For site-directed mutagenesis, oligodeoxyribonucleotides (mutagenesis oligomers) are synthesized with a sequence complementary to stretches of 15 bases on either side of the mutation site as shown in the following diagrams, wherein N indicates complementary bases and M indicates the nucleic acid to be inserted, deleted or substituted. Insertions or deletions should be made in groups of 3 in order to retain the downstream portion of the gene in phase.

For deletions:	oligomer DNA	(N)15 (N)15
	vector DNA	(N)15 (M) (N)15
For substitutions:	oligomer DNA	(N)15 (M1) (N)15
	vector DNA	(N)15 (M2) (N)15
For insertions:	oligomer DNA	(N)15 (M) (N)15
	vector DNA	(N)15 (N)15

M1 indicates a base or an oligomer which is not complementary to the base or oligomer M2. Here, M1 is the desired mutant sequence. Ordinarily, oligomers are also prepared that act to make more than one type of mutation at a time.

The antisense oligomer for the exemplary mutants are as shown in the following Table:

TNF- alpha Analogue Primer

Trp 28 - > Phe 28	PGGC CCG GCG GTT CAG AAA CTG GAG CTG CCC CTC OH
Trp 114 - > Tyr 114	PATA GAT GGG CTC ATA GTA GGG CTT GGC CTC AGC OH
DELTA Val 150 - Leu 157	PGTT GGA TGT TCG TCC TCC TCA CTG CCC AGA CTC GGC OH
DELTA Lys 11 - Arg 31	PGAG GGC ATT GGC CCG GTC ACT CGG GGT TCG OH
Phe152 Ser Ser Ser Gly153	PCAG GGC AAT GAT CCC ACT ACT ACT AAA GTA GAC CTG CCC OH
Leu26 Ser Ser Ser Gln27	PGCG GTT CAG CCA CTG ACT ACT ACT GAG CTG CCC CTC AGC OH

These primers are synthesized by conventional methods. For use in the mutagenesis procedure, 10 pmoles of the oligomers or lac primer 5'-CTTTTCCCAGTCACGAC-3' are each phosphorylated for 30 min at 37o C. in 10 mu l of 50 mM Tris-HCl (pH 7.5) containing 0.1 mMEDTA, 10 mM MgCl2, 10 mM dithiothreitol, 0.1 mM ATP and 2 U of T4 polynucleotide kinase. For use as probes (see infra), 2 pmoles of the synthetic oligonucleotides are phosphorylated as above except that 0.1 mM ATP was supplemented with 1 mu M gamma -<32> p ATP (Amersham). Specific activities are routinely higher than 5 x 10<6 > cpm/pmole of oligonucleotide chain.

Hybridization of each oligomer and the lac primer to the single-stranded DNA from phage M13 mp8/TNF, followed by primer extension, results in the formation of partial heteroduplex DNAs, one strand of which contains the DNA.

For partial heteroduplex formation, single-stranded M13 mp8/TNF DNA (300 ng) is heated to 80o C. (2 min), 50o C., (5 min), and room temperature (5 min) in 20 mu l 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 50 mM NaCl, containing 1 pmole each of phosphorylated oligomer and primer (added as aliquots from the kinase reaction). Primer extension is started by the addition of 30 mu l 50 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 12 mM MgCl2, 10 mM dithiothreitol, 0.7 mM ATF, 0.07 mM dATP, 0.2 mM each of dGTP, dTTP, dCTF, and containing 2 U E. coli DNA polymerase I, large fragment and 20 U T4 DNA ligase. After 30 min at room temperature, reaction mixtures are incubated for 4 hr at 37o C. followed by overnight incubation at 4o C. Aliquots are phenol extracted and DNA is precipitated with ethanol and dissolved in 15 mu l of water. DNA in these aliquots is used to transform E. coli JM103.

The lac primer hybridizes to the phage at a location 5, to the oligomer. Primer elongation stabilizes the heteroduplex structure. The oligomer and primer are enzymatically phosphorylated to allow the T4 DNA ligase to join connecting DNA chains.

Phenol extracted heteroduplex DNA from aliquot C (10 mu l) is added to 10 mu l 0.06M Na-acetate pH 4.5, 0.6M NaCl, 0.6 mM ZnCl2, and containing 200 U S1 nuclease. After incubation at 37o C. for 5 min, yeast tRNA (5 mu g) is added and nucleic acids are recovered by phenol extraction and ethanol precipitation. Using the same S1 conditions, 30 ng of single-stranded M13 mp8 DNA (about 10,000 plaque-forming units) yields less than 100 plaques in a DNA transformation assay, whereas the same amount of RF-DNA. retains more than 80 percent of its transforming properties. S1-treated DNA is used to transform E. coli JM103 and the resulting phage analyzed by in situ plaque screening.

Bacterial plates (15-cm diameter) containing several hundred recombinant M13 phage plaques are screened by in situ plaque hybridization (Benton et al., 1977, "Science" 196: 180-182) for both the parental and the mutated genotype using the appropriate labelled oligomers on separate sets of filters (about 10<6 > cpm per filter). Hybridization is overnight at 50o C., 40 percent formamide, 5 x SSC. Filters are washed at 45o C., 2 x SSC, 0.02 percent sodium dodecyl sulfate, air-dried, and exposed to X-ray film at - 70o C. using an intensifying screen.

It will be necessary to vary the stringency of the hybridization procedure (by altering the concentration of SSC) in order to resolve the hybridization of oligomer to the mutant DNA strand (a perfect complement) as opposed to hybridization to the starting DNA; each mutant will vary in its ability to hybridize depending upon the nature and number of bases substituted, deleted or inserted. For example, detecting a mutation in a single base will require high stringency conditions to discriminate between mutant and unmutated parental DNA where the mutation is minor, e.g. deletion of a codon or substitution of 1-3 bases, the hybridization probe should be smaller than the mutating oligomer. Typically this will be a probe of about 14 to 20 bases. The task of screening mutant deletions is facilitated by the use of a probe containing or constituting the deleted sequence to assay for loss of the sequence. If this probe fails to hybridize to DNA from a selected plaque one can conclude that the desired loss of the target sequence has occurred.

A plaque that hybridizes with the labelled oligomer is picked and inoculated into E. coli JM103. Single-stranded (ss) DNA is prepared from the supernatant and double-stranded (ds) DNA is prepared from the cell pellet. The ssDNA is used as a template for the dideoxy sequencing of the clone using the M13 universal primer or a synthetic oligomer complementary sequences located 3' of the mutated region of the tumor necrosis factor DNA. Dideoxy sequencing confirms that the recovered plaque contains the mutant DNA. Such phage is designated M13 mp8/TNFmtnt.

M13 mp8/TNFmtnt is digested with EcoRI and HindIII and the TNF variant-encoding fragment recovered. pTrpTNF is digested with EcoRI and HindIII and the vector fragment recovered. The fragment encoding the variant is then ligated to the vector fragment and the ligation mixture used to transform E. coli W3110, NL106, or 294 (ATCC 31446). The variant TNF is recovered by gel electrophoresis. A comparison of this gel with that obtained upon the parallel electrophoresis and staining of pTNFtrp-transformed E. coli protein shows a band migrating at approximately 17,000 daltons representing the variant protein. The amino acid sequence of the purified variant is confirmed. Residual cytotoxicity of the analogue is determined in the TNF cytotoxicity assay. Substantially non-cytotoxic analogues are assayed for antagonist activity by TNF composition as described above. Analogues that contain less than about 10% of the cytotoxic activity of TNF on a molar basis and which are able to neutralize at least about 20% of TNF cytotoxic activity on a molar basis are selected for analysis in in vivo experimental models.

EXAMPLE 3

Use of Neutralizing TNF- alpha Antibody to Suppress Mixed Lymphocyte Reaction

In the following study, human TNF- alpha and TNF- beta were obtained in > 98% purity from recombinant cell culture (rHuTNF- alpha and beta) rHuTNF- alpha was mature and contained 7.6 x 10<7 > U/mg protein as determined by the L cell assay described in Aggarwal et al., 1984, Thymic Hormones & Lymphokines, Goldstein, Ed. Plenum Publishing p. 235. The rHuTNF- beta formulation was of approximately the same purity and was assayed in the same fashion. rHuTNF- beta was the unglycosylated His24 N-terminal species. Rabbit polyclonal anti-rHuTNF- alpha antisera was obtained as described in Example 1. Blood drawn from healthy donors was heparinized, diluted with an equal volume of saline, layered on Ficoll-Hypaque gradients (Sp. gr. 1.08) which were centrifuged at 400 x g for 40 min. at room temperature. PMBC (peripheral blood mononuclear cell) isolated at the plasma-Ficoll interface were separated and washed three times in cold Hank's balanced salt solution (Grand Island Biological Co., Grand Island N.Y., (Gibco)). The final cell pellet was resuspended in complete medium and counted. Cell viability was >/= 95 percent as determined by trypan blue exclusion. RPMI 1640 medium (Gibco) was supplemented with 10 percent heat-inactivated pooled human AB serum (Peninsula Memorial Blood Bank, S. San Francisco, Calif.), 2 mM L-glutamine, 0.1 mM non-essential amino acids, 10 mM Hepes buffer (Gibco), 5 mu g/ml garamicin (Schering Corp., Kenilworth, N.J.) 500 U/ml penicillin, 500 mu g/ml streptomycin (Gibco) and 5 x 10< - 5 > M beta -mercaptoethanol (complete medium).

Mixed Lymphocyte Cultures

The proliferative response of the PBMC to allogeneic stimulation was determined in the one-way mixed-lymphocyte reaction (MLR) performed in replicates in microtiter plates as detailed previously (Shalaby et al., 1983, "Cell. Immunol." 82:269). Briefly, 10<5 > responder cells in 200 mu l of complete medium were co-cultured with either mitomycin C treated autologous cells (control cultures) or mitomycin C treated allogeneic cells (stimulated cultures) in replicates of three to six cultures. The treatment of PBMC with mitomycin C (Sigma Chemical Co., St. Louis, Mo.) was accomplished as described previously (Shalaby et al., 1983, "Cell Immunol." 82:269). Lymphokine effects were tested in cultures prepared in parallel. Cultures were incubated for 7 days. Six hr prior to harvesting, the cultures were pulsed with 2 mu Ci/well of [<3> H]-thymidine (40-60 Ci/mmol) (Amersham, Arlington Heights, Ill.) and processed with a multiple automated sample harvester onto glass-fiber discs. The discs were allowed to dry and [<3> H]-thymidine incorporation was determined using a Beckman scintillation spectrometer Model LS6800. Data were calculated as net counts per min (cpm) (mean cpm stimulated cultures minus mean cpm control cultures). Consistently, the mean <3> H-thymidine incorporated by control cultures was >/= 1.5 x 10<3 > cpm. Statistical analysis was performed using the paired-comparison (one-sample) student t test.

PBMC responder cells were incubated with mitomycin C-treated allogeneic cells in the absence (control) or presence of 1000 U/ml of recombinant human IFN-alpha 2/1, rTNF- alpha or rTNF- beta . <3> H-Thymidine incorporation was determined after seven days of incubation. Data are presented as mean cpm of 4-6 cultures +/- SE. The cpm of responders incubated with autologous stimulators were 2500 cpm or less.

TABLE 1

The effect of rHuIFN alpha 2/1, rHuTNF- alpha , and rHuTNF- beta on the proliferative response in MLR.

Culture	<3> H-thymidine incorporated (Cpm x 10< - 3 > 4 +/- SE)			
Condition	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Control	76 +/- 6	17 +/- 3	30 +/- 4	9 +/- 2
rHuIFN- alpha 2/1	4 +/- 1	2 +/- 0.3	22 +/- 4	2 +/- 0.4
rHuTNF- alpha	97 +/- 5	31 +/- 2	108 +/- 10	60 +/- 10
rHuTNF- beta	87 +/- 3	42 +/- 5	56 +/- 8	37 +/- 5

Other studies in which rHuTNF- alpha and rHuTNF- beta were added to the PBMC cultures at different times after the start of incubation with allogeneic cells demonstrated that rHuTNF- alpha produced maximal <3> H-thymidine incorporation only when added at the time the cultures were established, whereas rHuTNF- beta produced no change in enhancement whether added at 0, 3, 4 or 5 days of incubation. At day 6 no effect was observed. It was apparent that rHuTNF- alpha and rHuTNF- beta were enhancing the proliferative response of PMBC to an antigenic stimulus and that suppression of this response would be useful in suppressing undesirable immune inflammatory conditions. In order to determine whether a rHuTNF antagonist would function in suppression of PMBC proliferation, additional experiments were performed using specific rabbit antibodies against rHuTNF- alpha . Aliquots of rHuTNF- alpha (1000 U/ml) were incubated in media only or in media containing either specific polyclonal antisera adequate for the neutralization of 1000 U rHuTNF- alpha or normal rabbit serum used at a final dilution similar to that for the neutralizing antiserum. After 2 h of incubation at 37o C., these aliquots (at the same dilution) were added to MLR cultures at desired concentrations and also tested in the L cell bioassay to confirm the neutralization of rHuTNF- alpha cytotoxic activity. The results of (Exp. 1, Table 2 below) demonstrate that rHuTNF- alpha which had been neutralized with antibodies failed to cause an enhancement of MLR, unlike the phenomenon observed when unneutralized rHuTNF- alpha alone was added to cultures. The presence of specific antibodies alone did not influence the reaction in this particular experiment (Exp. 1, Table 2).

TABLE 2

The specificity of rHuTNF- alpha induced effect and the impairment of proliferative activities by rabbit antibodies against rHuTNF- alpha

Culture	<3> H-thymidine incorporated (Cpm x 10< - 3 > 4 +/- SE)				
Condition	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
Control	21 +/- 3	14 +/- 1	40 +/- 10	20 +/- 1	138 +/- 26
rHuTNF- alpha	86 +/- 8	27 +/- 2	64 +/- 8	79 +/- 14	173 +/- 11
Ab + rHuTNF- alpha	31 +/- 5	16 +/- 3	0.5	0.5	69 +/- 11
Ab only	15 +/- 8	1.2	0.5	1.5.	71 +/- 6
NRS	48 +/- 7	12 +/- 3	N.D.*	N.D.*	143 +/- 11

n*Not determined -

In repeat experiments, it was noticed that the addition of antibodies against

rHuTNF- alpha to cultures of MLR in some cases completely abolished <3> H-thymidine incorporation (Exp. 2, 3, and 4, Table 2) or caused a 50 percent inhibition of the reaction (Exp. 5, Table 2). These results raised the possibility that antibodies against rHuTNF- alpha may interfere with the synthesis of lymphokine(s) e.g., interleukin-1 (IL-1) and IL-2, that regulate the proliferative activities of responder cells in MLR (Muller, G. (ed) Immunol. Rev. 51, 1980). To test this hypothesis, cultures of MLR were established with and without the addition of 1000 U/ml rHuTNF- alpha , or specific antibodies. After 24 h of incubation, supernatants were collected and tested for IL-1 activity by the murine thymocyte assay (Mizel, 1981, In: Manual of Macrophage Methodology, Herscowitz et al., Eds.). The results of these experiments demonstrated supernatants of rHuTNF- alpha treated cultures contain an increased level of IL-1 activity and that the addition of specific antibodies against rHuTNF- alpha causes a 40-60 percent suppression of IL-1 activity in MLR supernatants.

EXAMPLE 4

Suppression of Graft versus Host Reaction In Vivo by Administration of Anti-rHuTNF- alpha

The purpose of this study was to investigate the influence of administered rMuTNF- alpha and rabbit antibodies (RbAb) against rMuTNF- alpha on the development of graft-vs-host reaction (GVHR) in newborn randomly sexed BDF1 mice. The results of preliminary experiments indicate that the administration of rMuTNF- alpha can further enhance GVHR and, conversely, the administration of RbAb decreases the severity of GVHR as determined by the Simonsen spleen weight assay. (Simonsen, M. et al., 1959, In: Biological Problems of Grafting, F. Albert et al., editors Charles C. Thomas, Publisher, Springfield, Ill. 214).

The cloning and expression in E. coli of the CDNA for murine TNF- alpha have been reported (Pennica, D., et al., Proc. Natl. Acad. Sci. 88: 6060, 1985). The materials used were purified to greater than 98 percent purity. Titers of recombinant murine TNF- alpha (rMuTNF- alpha) were calculated on the basis of its cytotoxic activities as determined by the L cell bioassay (Aggarwal, op cit) and showed a specific activity of 7-8 x 10<7 > U/mg protein. Polyclonal antibodies against rMuTNF- alpha were generated in rabbits immunized with purified preparations of rMuTNF- alpha substantially as shown in Example 1 (Nedwin G. E. et al., J. Immunol. 135: 2492, 1985) and had a neutralization titer of 2.5 x 10<6 > U/ml as determined by bioassay results of neutralized rMuTNF- alpha activity.

Newborn BDF1 (C57BL/6 x DBA/2) litters with C57BL/6 (B6) mothers purchased from Simonsen laboratories (Gilroy, Calif.) were used. Nursing mothers were allowed access to Purina lab chow and water ad libitum and the newborns were put on study in 24-48 h of birth.

GVHR was measured by the Simonsen spleen weight assay op cit. Adult BDF1 and B6 spleens were made into a single cell suspension by teasing in Hanks balanced salt solution (HBSS). The red blood cells were lysed by hypotonic shock followed by centrifugation. Spleen cell pellets were suspended in HBSS, counted, and adjusted at the desired concentration. Spleen cells were injected

intraperitoneally into 24-48 hrs. old BDF1 newborns in the following experiments. The various groups of newborns were litters injected with HBSS, litters injected with adult syngeneic BDF1 spleen cells, litters injected with adult semi-allogeneic B6 spleen cells, litters injected with either of the spleen cells in combination with rMuTNF- alpha or antibodies against rMuTNF-alpha and litters injected with rMuTNF- alpha or antibodies only. The protocol for each litter consisted of the i.p. injection of about 2.5 x 10<6 > spleen cells. The amount of rMuTNF- alpha was 2000 units per animal administered in 0.1 ml of HBSS. The amount of rabbit antisera used (unfractionated) contained 2000 neutralizing units of antibodies against rMuTNF- alpha , this being diluted into 0.1 ml of HBSS for injection. A 2000 neutralizing unit dosage of antisera was mixed with spleen cells and then immediately injected. Then the same dose (2000 neutralizing units) was administered by i.p injection (without spleen cells) on days 1, 2, 3, 5 and 8. On day 10 all animals were sacrificed and their spleens examined. The animals weighed about 31/2-6 grams upon sacrifice, but their weight increased rapidly during the 10 days of the study due to normal growth. Thus, a suitable intraperitoneal dose in this experiment ranged from about 0.25 x 10<6 > to about 3 x 10<6 > neutralizing units/Kg/24 hours.

Spleen indices were calculated (Klein, J. and Park, J. M., J. Exp. Med. 137: 1213, 1973) and the data are expressed as mean spleen indices (MSI) which was obtained by averaging the spleen indices of at least 6 mice in each group.

In an initial experiment we tested the number of adult spleen cells required for the induction of GVHR in newborns. The results indicated that 2.5 x 10<6 > cells were adequate for causing a GVHR and that the severity of the reaction was only slightly increased by injection of an intermediate or a higher number of cells (10-25 x 10<6 > cells). The results of five independent experiments, summarized in Table 3, show that enhancement of GVHR by rMuTNF- alpha injection was not observed consistently (marked enhancement in Exp. 2, and only a slight enhancement in Exp. 3, Table 3). However, a consistent suppression of GVHR was observed in mice receiving injections of antibodies against rMuTNF- alpha (Exp. 1,4, and 5, Table 3). Rabbit anti-rMuTNF- alpha or rMuTNF- alpha injected in the absence of exposure to B6 cells had no effect.

TABLE 3
The Influence of rMuTNF- alpha and Antibodies against rMuTNF- alpha on GVHR

	Mean Spleen Indexes				
Treatment	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
HBSS	0.53	0.43	0.52	ND	ND
rMuTNF- alpha	0.48	0.54	ND	ND	ND
BDF1 cells	0.45	0.47	0.44	0.53	0.52
B6 cells	1.51	1.12	1.36	1.86	2.19
B6 cells + rMuTNF- alpha	1.50	1.41	1.50	1.85	ND
B6 cells + Antibodies	1.17	ND	ND	1.39	1.29

nND = Not determined -

CLAIMS: We claim:

[*1] 1. A tumor necrosis factor-alpha (TNF-alpha) antagonist comprising an antibody which neutralizes cytotoxic activity of human TNF-alpha.

[*2] 2. The TNF-alpha antagonist of claim 1 wherein said antibody comprises a monoclonal antibody.

[*3] 3. The TNF-alpha antagonist of claim 2 wherein said monoclonal antibody is an IgG antibody.

[*4] 4. The TNF-alpha antagonist of claim 2 wherein sad monoclonal antiody is an IgM antibody.

[*5] 5. The TNF-alpha antagonist of claim 1 wherein said antibody comprises a murine antibody.

[*6] 6. The TNF-alpha antagonist of claim 1 wherein said antibody comprises a rabbit antibody.

[*7] 7. The TNF-alpha antagonist of claim 1 wherein said antibody comprises a monovalent antibody.

[*8] 8. The TNF-alpha antagonist of claim 7 wherein said monovalent antibody comprises a fab fragment containing a variable region of the antibody.

[*9] 9. A TNF-alpha antagonist comprising an anti-TNF-alpha antibody which blocks cytotoxic activity of human TNF-alpha.

[*10] 10. A TNF-alpha antagonist comprising a monoclonal antibody which neutralizes cytotoxic activity of human TNF-alpha, wherein said antibody is generated using a human TNF-alpha protein which, when in a purified form, migrates as a single band on a SDS-PAGE gel to a molecular weight of about 17,000 daltons.

[*11] 11. A TNF-alpha antagonist consisting of a monoclonal anti-TNF-alpha antibody which blocks cytotoxic activity of TNF-alpha on murine L-929 cells in vitro.

[*12] 12. A composition comprising the TNF-alpha antagonist of claim 1 and an isotonic carrier.

[*13] 13. A composition comprising the TNF-alpha antagonist of claim 9 and an isotonic carrier.

[*14] 14. A composition comprising the TNF-alpha antagonist of claim 10 and an isotonic carrier.

[*15] 15. A composition comprising the TNF-alpha antagonist of claim 11 and an isotonic carrier.